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A MANUAL

OF

BACTERIOLOGY

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BY

HERBERT U. WILLIAMS, M.D.

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WITH SEVENTY-EIGHT ILLUSTRATIONS.

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PREFACE.

In this manual the writer has endeavored to describe the laboratory technique which the beginner must follow, and at the same time to give a concise summary of the facts in bacteriology most important to the physician. In preparing a work of this character, which claims to be nothing more than a compilation, the standard text-books were necessarily consulted freely. On account of the need for brevity it has, in most cases, been impossible to mention authorities.

The writer is glad to have this opportunity to acknowledge his obligation to the works of Sternberg, Flügge, Günther, Eisenberg, Abbott, Muir and Ritchie, Vaughan and Novy, and McFarland; and to numerous papers by Prof. Welch and others. It is thought that the chapters on Germicides, and Surgical Disinfection, by Drs. Thos. B. Carpenter and Chauncey P. Smith, will be useful not only for the information presented in them, but especially in correlating that information with the facts of bacteriology.

Buffalo, New York,
October, 1898.

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INTRODUCTION.

ANYONE who has not himself worked in a bacteriological laboratory finds it difficult to form a vivid conception of what bacteria are like, because among the familiar animals and plants there are none with which a close comparison can be made. Of the common organisms, perhaps ordinary yeasts and moulds are most like the bacteria. Yeasts and moulds, as everyone knows, grow on bread, cheese, meat, syrups and the like. They flourish in moist and dark places, as do mushrooms, puffballs and the other fungi. All these fungi, appearing so different in some respects, are alike in one particular, which is the absence of the green color that we are apt to think of as being the essential feature of vegetation. Plants that are green owe their color to a substance called chlorophyll. Upon the properties of this substance one of the most fundamental facts in biology depends. Under the influence of sunlight, by means of chlorophyll, plants are able to use as food the carbon dioxide which is always present in the atmosphere in small amounts. Although carbon dioxide is one of the most simple and stable of compounds, the union of its component elements is broken by the plant, and they are employed in the formation of other much more complex and unstable compounds, such as starch and cellulose, which enter into the plant structure. The work of plants, it will be noticed, is, in the main, precisely the reverse of that performed by animals. Animals take the unstable carbohydrates with high potential energy, such as starches and sugars, as food, and exhale the stable carbon dioxide from the lungs. At the same time the animal receives the benefit of the energy resulting from the oxidation of the carbohydrates, which may appear indirectly in the form of nervous or muscular activity or warmth.

Those plants that are devoid of chlorophyll are compelled to some extent to use the same kinds of food as animals. They are unable to decompose carbon dioxide (in most cases), and procure their nourishment from the dead or living bodies of other plants or animals. Since they have no chlorophyll, light is of no advantage to them, and is often a positive detriment. Bacteria contain no chlorophyll, and are usually classed with the fungi, which they resemble in their inability to decompose carbon dioxide and to use it as food.*

There is another well-known property, possessed by yeasts especially, which may be useful in explaining the work done by bacteria. It is a fact of every-day observation that, when yeasts grow in dilute solutions of sugar, alcohol and gas are formed. It not only appears that bacteria sometimes form alcohol and gas from sugar, but that with different kinds of bacteria and different kinds of food material a great number of substances are made, some of which are of immense importance in producing disease.

The extreme smallness of the bacteria prevents us from seeing them as individuals without the aid of the microscope, although great numbers of them taken together may form a plainly visible mass or growth. When they are examined with the microscope they appear as little round, rod-shaped or curved bodies, which may be likened to so many periods, dashes and commas. It is at once perceived that each bacterium is an individual by itself, and that it consists of a single cell, not of an aggregation of cells, as do most of the common plants and animals.

Under favorable conditions bacteria may be seen to multiply, one organism being divided by a partition into two parts, which separate and become two new organisms. The process is called *fission.*

---

* See Chapter I., Part II.

At times certain bacteria present little bright spots which enlarge, and from which the rest of the cell breaks away in fragments. The bright body that remains is called a *spore*, and has greater resisting power against injurious influences than has the fully developed organism. To this extent these spores are something like the seeds of higher plants. There are spores that can withstand boiling for hours, but fortunately that is not true, as far as we know, of the spores of any of the bacteria that produce disease. The earlier investigators observed the appearance of bacteria in nutrient infusions which they had endeavored to sterilize by heat. They looked upon this fact as indicating the possibility of *spontaneous generation*, and it furnished the chief support of that theory. Probably their fluids contained very resistant spores, and were in fact not sterile.

From these facts, a definition for bacteria may be formulated.

Bacteria (Greek βακτήριον, meaning a little stick) *are extremely minute, unicellular plants, which have no chlorophyll, and which divide by fission.* They are sometimes called *schizomycetes.* In every-day language they are known as *microbes*, and also as *germs.* They are generally classed with the fungi. In some respects they seem quite closely related to the algæ or simplest green plants, and, on other hand, they have strong points of likeness with some of the unicellular animals belonging to the infusoria.

Bacteria are divided into three great groups:

Micrococci, or cocci[*] (singular, coccus)—spherical forms.

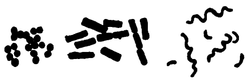
---

[*] **Pronounced koḱ-sī or koḱ-kē, preferably the former: see Webster's International, Century, and Standard Dictionaries, and Foster's and Keating's Medical Dictionaries. The writer knows of no authority for the prevailing pronunciation koḱ-kī.**

Bacilli (sing., bacillus)—long and straight, or rod-shaped bacteria.

Spirilla (sing., spirillum)—consisting of spiral filaments like the turns of a corkscrew, or parts of spirals shaped like commas.

The extreme smallness of the bacteria is hard of comprehension. We may say, of most of them, that from 5000 to 25,000 placed end to end would make a line about an inch in length. When one touches a growth of bacteria with the sterilized platinum wire and spreads the tiny portion that adheres to the wire upon a slip of glass, it is found upon examination with the microscope that the bacteria left on the glass may be compared to the stars in the sky, the sands on the shore, or any of the other standard similes for numbers that are nearly beyond computation.



Micrococci Bacilli Spirilla

FIG. 1.

It is well known that bacteria are present on most of the objects about us. They occur on the skins of men and other animals, as well as in the mouth, stomach and intestines, and on most of the surfaces of the body that open to the external world. They are found in the water of rivers, lakes, and in the ocean. They appear in the soil down to a depth of several feet. They float in the air, except at high altitudes and over the ocean. Investigators have even reported finding them fossilized, indicating, as we might expect, that they existed at remote periods in the earth's history. It has been humorously suggested that the imps that escaped from Pandora's box were in reality bacteria. But the vast majority of them are entirely harmless as far as we are concerned, and many of them are indispensable in maintaining the balance existing between the different kinds of living things.

Were it not for the putrefactive and nitrifying bacteria the dead bodies of plants and animals would lie practically unchanged where they fell, and the fertilization of the soil necessary for the life of most plants, by means of substances derived from them, would cease.

Some bacteria have been made to do work in industries, like the bacilli whose growth in cream imparts an agreeable flavor to the butter.

The study of bacteria has led to the understanding of many hitherto unexplained facts. The unaccountable development of a moist, brilliant red deposit on bread and other articles of food, which was formerly believed by the superstitious to be blood, deposited by some miraculous agency, we know to be due to the growth of a common organism (bacillus prodigiosus).

It seems that in some cases in which death was attributed to the suction of air into the veins, because air appeared to be present inside the heart, the air was in reality a gas, formed by certain bacilli that invaded the body just before or just after death (bacillus aërogenes capsulatus).

Woodhead tells us that some savages are in the habit of smearing the soil of certain localities upon their arrows for an arrow-poison, which is intelligible in the light of the fact that soil often contains the bacilli of tetanus (lockjaw).

The comparatively small number of species of bacteria that cause disease are the ones that interest us most, and are those which have been most carefully studied. The necessity that falls upon bacteria, in common with other fungi, to derive their food from organic matter makes it easy to understand that they should frequently exist as parasites upon living animals and plants. Pear-blight is caused by bacteria. We find that frogs, birds, cattle and a great number of animals besides men suffer from diseases produced by bacteria.

When bacteria are placed upon slips of glass they may be studied with the microscope while alive. Some of them when living are motionless; others wriggle vigorously. Some dart about like minnows in a stream, or they make their way slowly across the field of the microscope like a boat that is being sculled from the stern. By proper methods it can be shown that the movements are effected through one or more fine, hair-like processes, called flagella.

Oftener it is expedient to study them after drying them on slips of glass, when they may be made more conspicuous by giving them an artificial color (staining). Some of the substances of which bacteria are composed readily absorb certain dyes. For this purpose the aniline dyes are used, and their employment has been one of the important factors in making progress in bacteriology possible.

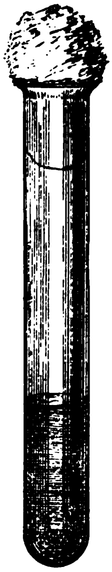


FIG. 2.—Test-tube containing Culture-medium.

With the microscope alone it is not usually practicable to distinguish accurately between various kinds of bacteria. Micrococci, for instance, which are, in reality, extremely different, may look very much alike. The differences are usually apparent when the bacteria are grown artificially. The cultivation is done for the most part in test-tubes containing some material which furnishes suitable food. The nutrient materials most used are meat-extract and peptone, which, dissolved with salt in water, constitute *nutrient bouillon*. Ordinary *gelatin*, or a vegetable gelatin called *agar-agar*, may be added to the bouillon when a solid *culture-medium* is desired. Before these substances can be used for the cultivation of bacteria all other bacteria which they might contain must be destroyed by heat.

When bacteria are to be conveyed from one tube to another, or from a tube to a glass slide, in order to examine them with the microscope, the manipulation is performed on a platinum wire fastened into a glass rod. The rules laid down for the management of the tubes and the platinum wire (pages 21 and 62) must be carefully followed. There is no danger in bacteriological work if the proper precautions are conscientiously observed; but carelessness may lead to disastrous and even fatal results, as has happened more than once.

Finally, the effects of bacteria in bringing about disease may be tested on the lower animals. The proof that a particular species of bacteria causes a particular disease cannot be considered complete unless the disease can be reproduced by introducing these bacteria into some animal.

The student who wishes to pursue bacteriological study in any direction farther than it is possible for the limits of a short manual to go, may, besides consulting the large text-books, obtain much assistance from periodical literature. The *Journal of Experimental Medicine*, published in this country, and the English *Journal of Pathology and Bacteriology* will give a great deal that is valuable.

A reading knowledge of German and French is very desirable. The *Centralblatt für Bacteriologie*, etc., a German weekly, and *Baumgarten's Jahresbericht der Mikroörganismenlehre* contain abstracts of most of the important researches made in all parts of the world.

PART I.

CHAPTER I.

EXAMINATION OF BACTERIA WITH THE MICROSCOPE, INCLUDING METHODS OF STAINING.

The Microscope.—The microscope consists of a tubular body which carries the optical parts, and which can be raised or lowered for focusing. The objectives should be three in number, and should be attached to the body by means of a triple nose-piece, which permits any objective to be turned into the optical axis as desired. The eye-piece slips into the upper and opposite end of the body or tube. The arrangements for focusing consist of a rack and pinion which accomplish the coarse adjustment, and a more delicate fine adjustment. The stage, upon which the objects to be examined are placed, has an opening in the middle. In this opening an iris diaphragm and Abbé condenser are inserted. The iris diaphragm enables one to alter the size of this opening as desired. Beneath the stage is a movable mirror, of which one side is plane and the other concave. All of these parts are supported on a short, heavy pillar which is fixed in the horseshoe-shaped base.

The essential parts of the microscope are, of course, the eye-piece (German, *Ocular*), and the objective. Objectives are given various names by different makers, for instance, A, B, C, etc., or 1, 2, 3, etc.; or they are named according to their focal distances, as ⅔ inch, ¼ inch, ½ inch, etc. In bacteriological work a rather "low power" ⅔ or ¾ inch objective, an ordinary "high power" $\frac{1}{4}$ to $\frac{1}{6}$ inch dry objective, and a high power $\frac{1}{12}$ inch oil-immersion objective are needed. The magnification with the $\frac{2}{3}$ or $\frac{3}{4}$ inch objective is about 75 to 100 diameters; with the $\frac{1}{4}$ to $\frac{1}{6}$ inch 300 to 500 diameters; with the $\frac{1}{12}$ immersion 750 to 1000 diameters. The magnification varies according to the eye-piece used, as well as with the objective. A 1 inch and $1\frac{1}{2}$ inch eye-piece (Zeiss No. 2 and No. 4) serve well for most purposes. The oil-immersion objective is used in the exami-

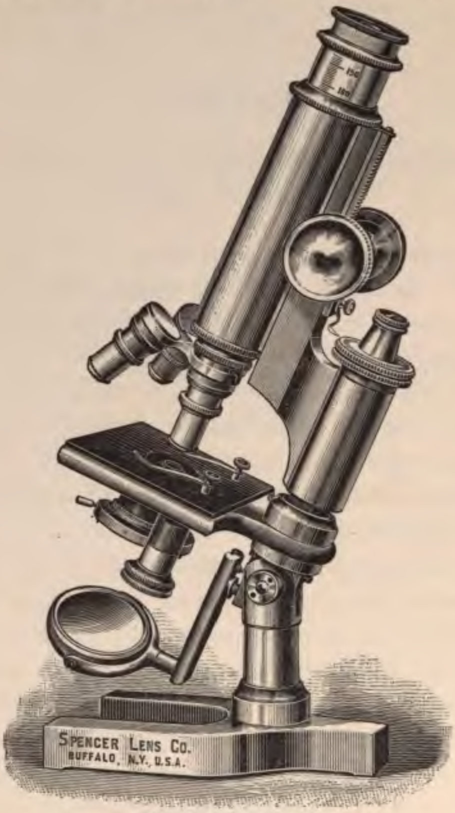

SPENCER LENS CO.
BUFFALO, N.Y., U.S.A.

FIG. 3.—Microscope.

nation of bacteria where a very high power is desired. A layer of thickened oil of cedar-wood is placed between the lower surface of the objective and the upper surface of the glass covering the object under examination. The oil must be wiped away from the surface of the objective when the examination is finished. For this purpose the soft paper sold by dealers in microscopical apparatus serves admirably. Care must be taken not to scratch the lower surface of this objective. Oil of cedar-wood furnishes a medium having nearly the same refractive index as the glass of the lens and the glass on which the object is mounted, and it obviates the dispersion of light which takes place when a layer of air is interposed between the objective and the object, as happens with the ordinary dry lens. This objective is used in connection with the Abbé condenser, which consists of two or three lenses combined so as to focus the rays coming from the plane mirror upon the object. The condenser gives a very intense illumination over a very small field. The condenser is not necessary excepting with the oil-immersion objective. If it is used with the other objectives the illumination must be regulated by lowering the condenser, closing the diaphragm more or less, and substituting the concave for the plane mirror. The eye-pieces are usually named arbitrarily, like the objectives. It is to be remembered that more depends upon securing a distinct picture than upon a very high magnification of the object.

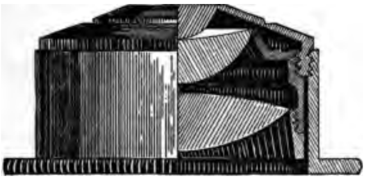


FIG. 4.—Abbé Condenser.

The microscope should be placed in front of the observer on a firm table. The observer should be able to bring the eye easily over the eye-piece when the tube of the microscope is in a vertical position. Daylight should be taken if possible. When artificial illumination is necessary, an ordinary lamp, a Welsbach burner or an incandescent electric light may be used. It is best to modify the artificial light by inserting a sheet of blue glass between the light and the mirror.

In order to focus upon any object, having first secured a satisfactory illumination with the mirror, it is best, beginning with the low power and using the coarse adjustment for focusing, to bring the objective quite close to the object, and then, with the eye in position, to raise the tube until the object comes into focus. The exact focusing is done with the fine adjustment. The observer should keep both eyes open when using the microscope, and should be able to use either eye at will.

All measurements of microscopic objects are expressed in terms of a micromillimeter. This is one-thousandth of a millimeter (.001 m.m.), which is about $\frac{1}{25000}$th of an inch. It is generally called a micron for short, and is denoted by the Greek letter μ. For example, $5\mu = .005$ m.m. $= \frac{1}{5000}$ inch.

The Preparation of Specimens of Bacteria for Examination with the Microscope.—The substance under examination is usually placed upon thin slips of glass called cover-glasses. The material is spread over the cover-glass by means of a platinum wire which has been fixed in a glass rod. Such a platinum wire is used constantly in doing bacteriological work. It is the tool by means of which one is able to handle bacteria with impunity. It serves in fact as a kind of additional finger. The platinum wire must be stiff enough not to bend too easily, and yet it should not be so large that it will not cool rapidly after heating. The wire may be straight throughout its length, or the tip may be bent to form a loop (German, *Oese*). It is well to follow, from the beginning, certain rules which make the use of the platinum wire safe and accurate. Every time it is taken into the hand and before using it for any manipulation it should be heated in the flame of a Bunsen burner or an alcohol lamp to a red heat; and always, after using and before putting it down, it should again be heated to a red heat. After the needle has become wet by dipping it in a fluid and is to be sterilized in the flame, it is necessary to avoid "sputtering" of the fluid by bringing the wet needle gradually to the flame, so as to dry the material adhering to it before burning it. This precaution is especially important when the wire has been dipped in milk or other substances containing oil. When the needle "sputters," as it is called, from too rapid heating, particles that have not yet been sterilized may be thrown some distance. On no account should the needle touch any object other than that which it is intended it should touch. With such a platinum wire, which has been properly sterilized, one can easily remove portions from a culture of bacteria, or from a fluid in which bacteria are supposed to be present. The glass rod in which the platinum wire is fixed should be held between the thumb and forefinger of the right hand like a pen. (For the manner of holding test-tubes, see page 62.)

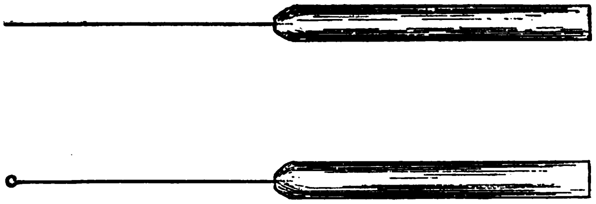


FIG. 5.—Straight Platinum Wire and Platinum Wire Loop.

The Hanging-drop.—Living bacteria may be studied with the microscope while suspended in some fluid substance. The needle having been sterilized in the flame and having been allowed to cool, a small portion of the culture or other material may be removed with it and deposited in the center of an ordinary cover-glass. When cultures on solid media are to be examined, a small particle may be mixed with a drop of sterilized water or bouillon. The cover-glass should have been carefully cleaned and sterilized over the flame. The cover-glass with the small drop of fluid material held in sterilized forceps is now to be inverted over a sterilized glass slide, which has a concavity ground in the middle of it. Around the concavity, the slide should be smeared with vaseline. In this manner a small air-tight chamber is made. This slide and cover-glass may be put upon the stage of the microscope. A good dry lens, if of sufficiently high power, is more convenient for examining the hanging-drop than an oil-immersion. If the latter be used, having placed a drop of cedar-oil on the center of the cover-glass, and a good light having been secured, the oil-immersion objective should be brought down upon this drop of oil. The beginner often experiences difficulty in focusing upon a hanging-drop. It is well to shut off most of the light by means of the iris diaphragm. Often it is well to secure the focus roughly upon the extreme outer edge of the chamber, or to find the edge of the drop of fluid with the low power and then to focus upon this edge with the oil-immersion objective. Above all things guard against breaking the cover-glass by forcing the objective down upon it. The motility of certain bacteria is one of the most striking phenomena to be ob-

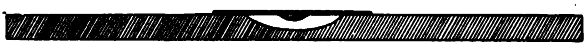


FIG. 6.—Diagram of the Hanging-drop.

served in the hanging-drop. It is not to be confused with the so-called "Brownian movement" which is exhibited by fine particles suspended in a watery fluid. It is well for the beginner to observe the character of the Brownian movement by rubbing up some carmine in a little water, and with the microscope to study the trembling motion exhibited by these particles of carmine. It will be noticed that, although the particles oscillate, no progress in any direction is accomplished unless there are currents in the fluid. Such currents might give rise to the impression that certain bacteria possessed motility when they were, in fact, powerless to move of themselves. In the hanging-drop the multiplication of bacteria can be studied, the formation of spores and the development of spores into fully formed bacteria. The hanging-drop has recently been put into service for the demonstration of the so-called Widal serum reaction with the bacillus of typhoid fever. Sometimes bacteria must be watched in the hanging-drop for hours, or even days, and it may be necessary to keep it at the temperature of the human body for this length of time. Various complicated kinds of apparatus have been devised for this purpose, but they are needful only with special kinds of work. When the hanging-drop preparation is no longer required, the slide and cover-glass should be dropped into a 5 per cent. carbolic acid solution and afterward sterilized by steam.

Cover-glass Preparations.—The study of bacteria with the microscope is for the most part done by means of smears made upon thin slips of glass. Such slips of glass are generally called cover-glasses. It is best to obtain the kind sold by dealers as No. 1, ¾ inch squares.

The cover-glass may be cleaned by immersion in a mixture of sulphuric acid and bichromate of potassium solution, and afterward washed thoroughly in distilled water, and finally in alcohol. A stock of clean cover-glasses may be kept in a bottle of alcohol.

Before using, the cover-glass is to be wiped clean with a piece of linen cloth. Whenever it is taken into the fingers it should be held by the edges, never by the flat surfaces. As far as possible it should be handled with the forceps. It can be used very conveniently in the form of forceps known as the Cornet forceps, or in the modification devised by Stewart. Bacteria may be placed upon the cover-glass by allowing the glass to fall upon one of the colonies of bacteria, on a gelatin or agar plate (see page 74), which will adhere to it in part, producing an "impression preparation" (German, *Klatschpreparat*). Such a preparation, after drying in the air, is to be fixed by passing it through the flame three times. (See below.)

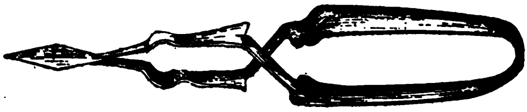


FIG 7.—Cornet Forceps for Cover-glasses.

Generally bacteria contained in fluids, like sputum, or taken from the surface of a culture, are smeared over the cover-glass by means of the platinum wire or loop. Such preparations are called smear, cover-glass, cover-slip, or film preparations. When the material to be spread is thick or very viscid, a small drop of distilled water must first be placed in the center of the cover-glass so as to dilute it. Beginners generally take too much material on the wire. As thin a smear as possible is made. It is allowed to dry in the air; this should occupy a few seconds. The drying

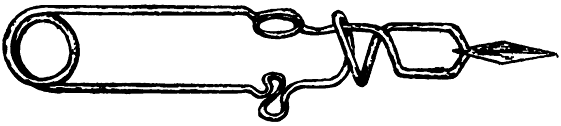


FIG. 8.—Stewart Forceps for Cover-glasses.

may be hastened by holding the forceps with the cover-glass a long distance above the flame, at a point where the heat would cause no discomfort to the hand. Having dried the preparation, it is to be passed through the flame of a Bunsen burner or alcohol lamp three times, taking about one second for each transit. The heat of the flame serves to dry the bacteria upon the cover-glass and fix them permanently in position. Such a preparation may be stained with one of the aniline dyes, and after washing in water and drying may be mounted, face down, in Canada balsam upon a glass slide. It makes a suitable object to be examined with the oil-immersion objective. The slide is a thin slip of glass, 3 inches by 1 inch, with ground edges.

The smear preparation may equally well be made directly upon the glass slide. The fixation in the flame must then occupy a longer time than with the small and thin cover-glass. Such preparations have the advantage that several may be made upon one slide, and that after staining them they may be examined in cedar-oil, with the oil-immersion lens, without the use of the cover-glass and Canada balsam.

When very virulent pathogenic bacteria are being handled, after fixation by heat upon the slide or cover-glass, the preparation may, if desired, be immersed in 1–1000 solution of bichloride of mercury long enough to kill the bacteria, without injuring the preparation or its staining properties.

Staining.—The staining of bacteria is done for the most part with the aniline dyes. The object of staining bacteria is to give them artificially some color which makes them distinct and easily visible without imparting this color to the substance or medium in which they are imbedded. The substances known as aniline dyes are derivatives of coal-tar, but not always of aniline. These dyes are of great importance in bacteriological work. Their number is very large, but only a few are in common use. It is important to have the purest, and those manufactured by Grübler are reliable.

It is simplest to classify the aniline dyes as acid or basic. Eosin, picric acid and acid fuchsin are acid dyes; they tend to stain the tissue diffusely. Fuchsin, gentian-violet and methylene-blue are basic dyes; they have an affinity for the nuclei of tissues and for bacteria; they therefore are the dyes used chiefly in bacteriological work. The other varieties may be employed as contrast-stains; another contrast-stain frequently used is Bismarck brown. It is best to keep on hand saturated solutions of the aniline dyes in alcohol, from which watery solutions may be made when needed by adding a few drops of the alcoholic solution to a small dish filled with water. The alcoholic solution is diluted about ten times, or so as to make a liquid which is just transparent in a layer about 12 m.m. thickness, after filtering.

Method of Staining Cover-glass Preparations.—(*a*) A smear preparation of bacteria having been made in the manner above described, and a watery solution of either fuchsin, gentian-violet or methylene-blue having been prepared, the cover-glass is to be dropped into a dish containing the dye, or the dye may be dropped upon the cover-glass held in the forceps.

(*b*) Allow the stain to act for about thirty seconds.

(*c*) Wash in water.

(*d*) Examine with the microscope in water directly or after drying and mounting in Canada balsam.

Preparations that are mounted at first in water may be made permanent by moistening the edge of the cover-glass so that it may easily be removed from the slide, then drying and mounting in Canada balsam. Cover-glass preparations which have been stained are examined with the oil-immersion objective, employing the plane mirror, having the iris-diaphragm open and the condenser close to the lower surface of the glass slide. The purpose is to obtain the most intense illumination possible over a small field. The rapidity and intensity of staining may be increased by warming slightly. The watery solutions of aniline dyes prepared as above described deteriorate in a short time, and it is best to prepare them freshly each time they are required. A very useful solution, which is permanent, is Löffler's alkaline methylene-blue:

Concentrated alcoholic solution of methylene blue,	30 c.c.
Potassium hydrate (caustic potash) 1-10,000 watery solution,	100 c.c.

Löffler's methylene-blue is a very useful stain for general purposes. It is perhaps more in use than any other formula for coloring the diphtheria bacillus.

Aniline-water Staining Solutions.—The intensity with which aniline dyes operate may be increased by adding aniline oil to the solution:

Aniline oil,	5 c.c.
Water,	100 c.c.

Mix, shake vigorously, filter; the fluid after filtration should be perfectly clear; add—

Alcohol,	10 c.c.
Alcoholic solution of fuchsin (or gentian-violet, or methylene-blue),	11 c.c.

Aniline-water staining solutions do not keep well, and need to be freshly prepared about every two weeks. The applications of the aniline-water stains will be given under separate headings. In general, however, they are employed where a stain of unusual power is required.

Gram's Method.—Cover-glass preparations, having been prepared and fixed in the usual manner (see page 24), are stained as follows:

(*a*) Stain in aniline-water gentian-violet solution, from two to five minutes. The intensity of the stain may be increased by warming slightly.

(*b*) Iodine solution, one and one-half minutes:

Iodine,	1 gram.
Potassium iodide,	2 grams.
Water,	300 c.c.

In this solution the preparation becomes nearly black.

(*c*) Wash in alcohol repeatedly; the alcohol becomes stained with clouds of violet coloring matter; the alcohol is used as long as the violet color continues to come away, and until the preparation is decolorized or has only a faint steel-blue color.

(*d*) When desired, the specimens may be stained, by way of contrast, with a watery solution of Bismarck brown or eosin.

(*e*) Wash in water, and examine either in water directly or after drying and mounting in Canada balsam. [A modification of this method, sometimes called the Gram-Günther method, differs from the preceding by using a 3 per cent. solution of hydrochloric acid in alcohol for ten seconds to hasten decolorization, washing in pure alcohol before and after the acid alcohol. Decolorization is more intense than by the Gram method; the diphtheria bacillus, which is stained by Gram's method, is decolorized by the Gram-Günther (Kruse).] The advantages of Gram's method are that certain bacteria are stained by it with great intensity and other bacteria are not stained at all. To some extent, then, it furnishes a means of diagnosis.

List of some of the important bacteria that are stained by Gram's method:

Staphylococcus pyogenes aureus,
Streptococcus pyogenes,
Micrococcus lanceolatus (of pneumonia),
Micrococcus tetragenus,
Bacillus of diphtheria,
Bacillus tuberculosis,
Bacillus of leprosy,
Bacillus of anthrax,
Bacillus of tetanus,
Bacillus aërogenes capsulatus,
Ray fungus of actinomycosis.

The following bacteria are not stained by Gram's method:

Gonococcus,
Bacillus of typhoid fever,
Bacillus coli communis,
The Spirillum of Asiatic cholera,
Bacillus pyocyaneus,
Bacillus of influenza,
Bacillus of glanders (bacillus mallei),
Bacillus of malignant edema,
Bacillus of Friedländer,
Diplococcus intracellularis meningitidis,
Bacillus proteus,
Spirillum of relapsing fever.

Staining the Bacillus of Tuberculosis.—A very large number of methods have been proposed for staining the bacillus tuberculosis, all of which depend upon the principle that, after adding to solutions of aniline dyes certain substances, like aniline-water, carbolic acid, solutions of ammonia or soda, the bacillus tuberculosis is stained with great intensity, and gives up its stain with difficulty. Solutions of acids will remove the stain from all parts of the preparation excepting from the tubercle bacilli, which retain the dye having once acquired it. The rest of the preparation may now be given a different color—contrast-stain. The process is most frequently used for specimens of sputum from cases of suspected pulmonary tuberculosis; it may be applied to other fluids and secretions equally well. Patients should be given minute instructions concerning the collection of sputum. The bottle used should be new, wide-mouthed, clean, and kept tightly stoppered with a clean cork. The patient should be cautioned against allowing the expectoration to get on the outside of the bottle. Probably whatever risk is incurred by those who examine sputum in large quantities comes chiefly from the outside of the bottle having been soiled with sputum containing tubercle bacilli. Often little white particles may be seen floating in the mucous portions of the sputum. These particles should be selected for the investigation. These specimens may be smeared on the cover-glass with the platinum wire, which is sterilized in the flame before and after using. The selection of the little white particles will be facilitated if the sputum be poured into a clean glass dish, which may be placed on a black surface. A form of porcelain dish is furnished by dealers, the bottom of which is black, and which is convenient for these manipulations. The smears must be made thin, or the subsequent decolorization, after staining, will not be uniform. It is hardly necessary to observe that the operator must be scrupulously careful not to contaminate the material under examination with any kind of extraneous matter. The cover-glass and slides which are used should be new, and should have been cleaned with bichromate of potassium and sulphuric acid (see page 23).

Concerning the danger of confusing tubercle bacilli with the bacilli of smegma, and the examination of discharges from the genito-urinary tracts, see page 23.

Occasionally certain spores, micrococci and horny epithelial cells are imperfectly decolorized, but their forms distinguish them from tubercle bacilli. Minute crystalline needles, which have a shape somewhat like that of bacilli, are often encountered in sputum, but their nature will be recognized after a little practice.

When the work is completed, the bottle containing the sputum should be sterilized by steam or boiling.

Gabbett's method is as simple and convenient as any. Only two solutions are required.

Ziehl's carbol-fuchsin:

Fuchsin,	1 gram.
Carbolic acid, pure,	5 c.c.
Alcohol,	10 c.c.
Distilled water,	100 c.c.

Gabbett's solution:

Methylene-blue,	1 to 2 grams.
25 per cent. watery solution of sulphuric acid,	100 c.c.

(*a*) The cover-glass preparation is to be made, dried, and fixed by passing through the flame three times.

(*b*) The carbol-fuchsin stain is applied from two to five minutes to the cover-glass, held in forceps or in a watch-crystal; it need not be warmed.

(*c*) Wash in water.

(*d*) Gabbett's solution is applied for one minute.

(*e*) Wash in water. The preparation should have a blue color. It may be examined in water directly or after drying and mounting in Canada balsam.

Gabbett's method has the advantage of decolorizing the preparation and staining the background with methylene-blue at the same time. Tubercle bacilli are colored a brilliant red; other bacteria and the nuclei of cells are colored blue.

Of the numerous methods of staining tubercle bacilli only a few others can be mentioned. Aniline-water fuchsin or gentian-violet, or carbol-fuchsin may be used. The intensity of the stain may be increased by warming the preparation, or by bringing it to the boiling-point, allowing the warm stain to act on the specimens for from three to five minutes; the preparation may also be left in the cold stain over night. Decolorization may be effected with a 25 per cent. solution of sulphuric acid used till the red color disappears, or a 30 per cent. solution of nitric acid, which operates very rapidly. If the red color persists after washing in water, dip in the acid again. After either acid the preparation is to be washed in alcohol until the last trace of the stain has been removed. An excellent decolorizing agent, and the one preferred by the writer, is a 3 per cent. solution of hydrochloric acid in alcohol, used for about a minute. With any of these acid solutions the decolorization can be accomplished more perfectly than with Gabbett's solution, where the operation of the decolorizing agent is masked. The contrast-stain may be omitted entirely if it is desired. A suitable contrast-stain after fuchsin staining is a solution of methylene-blue; after gentian-violet staining, Bismarck brown.

Those who have had experience in staining tubercle bacilli soon discover that the bacilli exhibit some differences in their resisting power to strong acids. One encounters occasionally bacilli that are perfectly stained side by side with others that are more or less completely decolorized. These facts show the necessity of practice with any method, and of exercising caution and judgment in making a diagnosis where the number of bacilli happens to be scanty. If tubercle bacilli are not found in the first preparation, other preparations should be made. Sometimes a large number of cover-glasses must be examined.

Various expedients have been devised to concentrate tubercle bacilli when only a small number may be present in a sample of sputum. In Biedert's method about 15 c.c. of sputum are mixed with 5 c.c. of distilled water, 4 to 8 drops of sodium hydrate solution are added, and the mixture is boiled. After boiling, add about 15 c.c. of distilled water. The mixture may be set aside in a conical glass for from twenty-four to forty-eight hours when the sediment may be collected, smeared on a cover-glass and stained for tubercle bacilli; or the sediment may be precipitated rapidly by the use of the centrifuge. The sediment will be found to have little adhesive power, and will not stick well to the cover-glass. It is convenient to save some of the original sputum and mix it with the sediment for this purpose.

Staining Bacteria in Tissues.—Pieces of organs about 1 cm. in thickness may be taken. Alcohol is the best agent for preserving them. The hardening will be completed in a few days. It is best to change the alcohol. The amount of the alcohol must be twenty times the bulk of the tissue to be preserved.

Ten parts formaldehyde with 90 parts water makes a good mixture for fixation; after twenty-four hours change to alcohol.

Imbedding in Collodion or Celloidin.—From alcohol the pieces of tissue are placed in equal parts of alcohol and ether twenty-four hours; thin collodion twenty-four hours; thick collodion twenty-four hours. The specimen is placed upon a block of wood and surrounded by thick collodion, and then inverted in 70 per cent. alcohol. The collodion makes a firm mass, surrounding and permeating the tissue, and permits very thin sections to be cut. The soluble cotton sold by dealers in photographers' supplies serves as well as the expensive preparation known as celloidin. To make collodion, dissolve it in equal parts of alcohol and ether. Soluble cotton is also called pyroxylin, and is a kind of gun-cotton.

Imbedding in Paraffin.—(*a*) Pieces of tissue 2 to 3 m.m. thick are to be placed in 95 per cent. alcohol for twenty-four hours.

(*b*) In pure chloroform six to eight hours.

(*c*) In a saturated solution of paraffin in chloroform two to three hours.

(*d*) In melted paraffin having a melting-point of 50° C., which requires the use of a water-bath or oven, one to six hours. The chloroform must be entirely driven off, and the tissue thoroughly infiltrated.

(*e*) Change to fresh paraffin for a few minutes.

(*f*) Finally, place the tissue in a small paper box and pour the melted paraffin about it. Harden as quickly as possible with running water. It is important to fix the piece of tissue in a suitable position, if the position is of importance, before pouring in the melted paraffin.

Sections of exquisite thinness may now be cut. The knife need not be wet. Paraffin imbedding is especially adapted for making serial sections.

In order to mount the sections, proceed as follows:

(*a*) Place the sections on a slide. Add a thin solution of gum arabic, upon which they float. Warm slightly, when the sections will flatten nicely. Drain off the superfluous gum solution, leaving the sections in their proper positions. Let them dry for some hours, and they will be firmly fastened to the slide.

(*b*) Dissolve out the paraffin in one of the numerous solvents (xylol half an hour or less).

(*c*) At this point the xylol should be washed off with alcohol, and

(*d*) The section is stained.

(*e*) Dehydrate in alcohol.

(*f*) Clear in some suitable agent, as xylol.

(*g*) Mount in balsam.

Section Cutting.—Cutting is best done with an instrument called a microtome. The tissues may be imbedded in collodion or paraffin; or they may be cut while fresh, after freezing; or tissues that have been hardened with formaldehyde may be cut after freezing. Bacteria stain admirably in frozen sections. For routine work collodion imbedding will be found as convenient a process as any. Paraffin imbedding gives the thinnest sections.

A microtome consists of a heavy, sliding knife-carrier, which moves with great precision on a level, and of a device for elevating through any desired distance the object which is to be cut, after each excursion of the knife. The thickness of the section will be the distance through which the object is raised. The knife is kept wet with alcohol during the cutting of collodion sections, otherwise it is left dry. The microtome is usually provided with a special form of knife. A razor will serve nearly as well, after having had the lower side ground flat. If a razor is used, a special form of razor-holder must be attached to the mi-

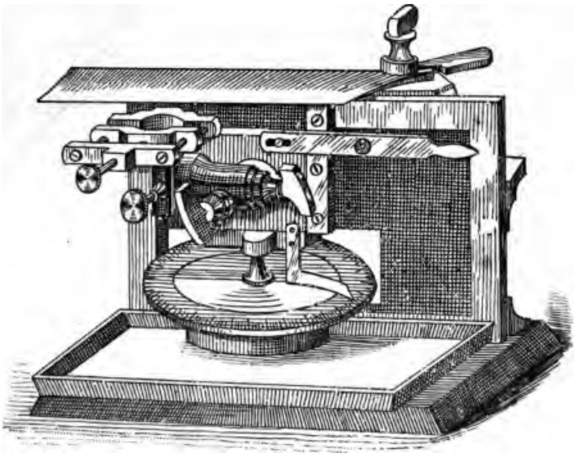


FIG. 9.—Schanze Microtome.

crotome to receive it. Above all, it is necessary that the knives should be kept in good condition. Only occasionally will they need honing, using a fine water-stone or Belgian hone. The movement in honing should be from heel to toe, always placing the back of the knife next the hone when turning. The knife should be stropped frequently. The leather of the strop should be glued to a strip of wood to make a flat surface. The movement in stropping should be from toe to heel. Sections should be cut to a thickness of not more than 25μ. Thinner sections are to be desired.

Staining of Sections.—A watery solution of one of the aniline dyes is used,—fuchsin, gentian-violet or methylene-blue,—made by adding a few drops of the alcoholic solution to a dish filled with water. Löffler's solution of methylene-blue serves very well.

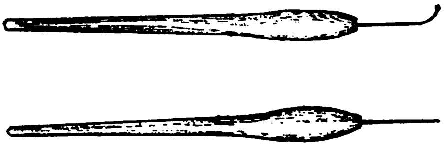


FIG. 10.—Needles for Handling Sections.

(*a*) Place the section in the staining solution from two to five minutes.

(*b*) Wash in water.

(*c*) Place in a watery solution of acetic acid, .1 per cent., for one minute.

(*d*) Alcohol, one to two minutes; change from one dish of alcohol to a fresh dish at least once. Touch the sections to blotting-paper to remove the superfluous alcohol.

(*e*) Xylol until clear; xylol is to be preferred to other clearing agents, like oil of cloves, most of which slowly remove aniline colors. It has the disadvantage of not clearing when the slightest trace of water is present; dehydration in alcohol must, therefore, be very complete. The section should be removed from the xylol as soon as it is cleared; otherwise wrinkling occurs.

(*f*) The section is placed upon a glass slide; a drop of Canada balsam is placed upon it and then a cover-glass. The Canada balsam should be dissolved in xylol.

The section is to be manipulated with straight or bent needles. The removal from xylol to the glass slide is managed best with a spatula or section-lifter.

By this process most bacteria are stained; also the nuclei of cells; frequently, also, certain granules contained within some cells (German, *Mastzellen*), which may easily be mistaken for bacteria by the inexperienced (basophile granules).

Gram's Method may be applied to the staining of sections of tissues as well as to smears upon cover-glasses.

(*a*) Place the section in aniline-water gentian-violet, one to five minutes.

(*b*) Iodine solution (see page 28), one and one-half minutes.

(*c*) Alcohol, until decolorized.

(*d*) Xylol.

(*e*) Mount on a slide in balsam.

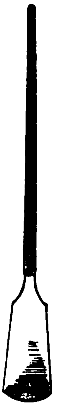


FIG. 11. Section-Lifter.

Weigert's Modification of Gram's Method, or Weigert's Stain for Fibrin.—(*a*) Place the section in aniline-water gentian-violet solution, five minutes or more.

(*b*) Wash briefly in water.

(*c*) Place the section upon a slide by means of a section-lifter; having straightened it carefully, absorb the water with blotting-paper.

(*d*) Iodine solution (see page 28), one to two minutes.

(*e*) Absorb the iodine solution with blotting-paper.

(*f*) Add aniline oil, removing it from time to time with blotting-paper, and adding fresh aniline oil until the color ceases to come away. (Aniline oil serves in this connection both to decolorize and to dehydrate. It absorbs the water rapidly and efficiently. However, on account of its decolorizing tendency, it must be removed before the specimens can be mounted permanently.)

(*g*) Add xylol; remove it with blotting-paper; and add fresh xylol several times, in order to extract the last trace of aniline oil.

(*h*) Mount in Canada balsam.

This method is more convenient for the staining of sections than the Gram method. The results, however, are essentially the same as far as the bacteria are concerned; fibrin and hyaline material are stained blue, bacteria violet. It is often impossible to decolorize the nuclei completely without decolorizing the bacteria also. The parts of the nuclei which remain stained often present pictures that resemble bacteria, and which may lead to error if not recognized. Basophile granules also retain the stain, as do the horny cells of the epidermis. These remarks apply also to Gram's method, except as regards fibrin. Very beautiful preparations can be obtained according to this or the Gram method when the sections have previously been stained in carmine; the nuclei will then be colored red, bacteria violet.

Tubercle bacilli may be *stained in sections* as follows:

(*a*) Use carbol-fuchsin, or aniline-water gentian-violet for one to two hours with very gentle warming, or over night without warming.

(*b*) Wash in water.

(*c*) Decolorize with some one of the decolorizing agents mentioned in connection with the staining of tubercle bacilli in cover-glass preparations, preferably 3 per cent. hydrochloric acid alcohol. Decolorization must be continued until the red color has disappeared, which requires one-half to several minutes.

(*d*) Wash in alcohol.

(*e*) Wash in water.

(*f*) Use hematoxylin as a contrast-stain for fuchsin preparations, and carmine for gentian-violet preparations. (It is better to stain with carmine first of all and before staining the bacilli. The carmine is not affected by the subsequent treatment.)

(*g*) Wash in water.

(*h*) Alcohol.

(*i*) Xylol.

(*j*) Balsam.

Nuclear stains, which may be used as contrast-stains for sections:

DELAFIELD'S HEMATOXYLIN.

Hematoxylin crystals,	4 grams.
Alcohol,	25 c.c.
Ammonia alum,	50 grams.
Water,	400 c.c.
Glycerin,	100 c.c.
Methyl alcohol,	100 c.c.

Dissolve the hematoxylin in the alcohol, and the ammonia alum in the water. Mix the two solutions. Let the mixture stand four or five days uncovered; it should have become a deep purple. Filter and add the glycerin and the methyl alcohol. After it has become dark enough, filter again. Keep it a month or longer before using; the solution improves with age. At the time of using, filter and dilute with water as desired.

BORAX-CARMINE (*Grenacher*).

Carmine,	2.5 grams.
Borax,	4.0 grams.
Alcohol (70 per cent.),	100.0 c.c.
Water,	100.0 c.c.

Rub the carmine and borax together. Dissolve them in the water, which should be hot. The alcohol may be added when the mixture is cold. The stain will be available after two weeks, but improves with age. Sections are to be left in the stain fifteen minutes or longer. In all cases carmine staining is to be finished with the use of acid alcohol, which differentiates the elements.

ACID ALCOHOL.

Alcohol (70 per cent.), ...	100 c.c.
Hydrochloric acid (strong), ...	1 c.c.

Sections stained in carmine are placed in acid alcohol for a few minutes. They acquire a brilliant scarlet color. When used as a contrast-stain for tissues containing bacteria, it is best to use it before staining the bacteria, which might be decolorized by the acid alcohol.

Staining of Spores.—The method is applicable to cover-glass preparations which may be prepared in the usual way from material supposed to contain spores.

(*a*) After drying the smear on the cover-glass, and fixation with heat by passing through the flame three times, use as a stain aniline-water fuchsin or gentian-violet.

(*b*) Heat until the preparation begins to boil; remove for a minute; heat again and again remove; repeat this process six times.

(*c*) Wash in 3 per cent. hydrochloric acid alcohol one minute.

(*d*) Wash in water.

(*e*) Stain with watery solution of methylene-blue half a minute.

(*f*) Wash.

(*g*) Dry.

(*h*) Xylol.

(*i*) Balsam.

The spores are intensely stained by the fuchsin. The stain is removed from everything except the spores by the acid alcohol. The methylene-blue solution stains the bodies of the bacteria, the spores remaining brilliant red. There are various other methods for staining spores, but this procedure gives good results. The principle is the same as in staining the tubercle bacillus, except that more pains are needed to impregnate spores with the dye.

Staining of Flagella.—Flagella are among the most difficult of all objects to stain. The best-known method is that of Löffler. It is important to use young cultures, preferably on agar.

(*a*) A small portion of the culture is mixed on a cover-glass with a drop of water. The preparations must be exceedingly thin. The mixing must be done with care in order not to break off the delicate flagella.

(*b*) After drying, fixation is effected by passing through the flame three times.

(*c*) The essential point in this method is the use of a mordant as follows:

Tannic acid, ...	2 grams.
Water, ...	8 c.c.
Saturated solution of ferrous sulphate, . . .	5 c.c.
Saturated alcoholic solution of fuchsin, . . .	1 c.c.

This solution is filtered and a few drops are placed on the cover-glass; it is then left for one minute, warming slightly.

(*d*) Wash in water.

(*e*) Stain with aniline-water fuchsin.

(*f*) Wash in water.

(*g*) Dry.

(*h*) Mount in Canada balsam.

(According to Löffler, certain bacteria require the addition of an acid solution, and certain others an alkaline solution, but many observers consider this unnecessary.)

CHAPTER II.

STERILIZATION.

By sterilization is meant the killing of all microörganisms found on any body or substance. It is possible to sterilize objects by the use of bichloride of mercury (corrosive sublimate), carbolic acid and other chemical agents. Sterilization is usually accomplished by heat. The most effective sterilization is that done by steam and by boiling; they are not, however, suitable for all kinds of material.

The Naked Flame of the Bunsen burner or the alcohol lamp is used largely for the sterilization of small articles. It is evident that no more efficient way of sterilization could be devised than by burning objects, or subjecting them to a red heat. The uses of this method will at once suggest themselves; for instance, surgical dressings that have become soiled with discharges and similar materials can be most easily disposed of by simply burning them up. In laboratory work the flame is constantly employed for the sterilization of the platinum wire, forceps, pipettes and cover-glasses; occasionally test-tubes are sterilized in this manner.

Hot-Air Sterilization.—Hot air, at a temperature of 150° C., or higher, maintained for an hour, is very valuable for some materials although less effective than steam. It has been found that the spores of certain bacteria are not killed even by exposure to this temperature, but it is sufficient for ordinary conditions. Hot-air sterilization is employed for glassware such as Petri dishes, flasks and test-tubes. The flasks and test-tubes are generally plugged with raw cotton.

The sterilization should change the cotton to a light brown color, but it should not be scorched to a dark brown. Glassware should be placed within the sterilizer when cold, and after heating should be allowed to cool gradually in order to avoid breaking. Hot-air sterilization is never used for culture-media, but all kinds of metal instruments, which the heat would not damage, may be sterilized in this fashion.

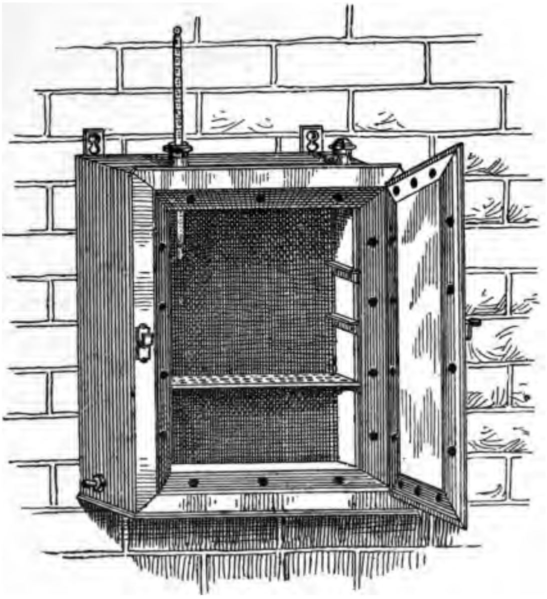


FIG. 12.—Hot-Air Sterilizer.

The hot-air sterilizer is a box made of sheet-iron, the walls being double, with an air-space between them. On one side is a door. There are openings at the top to secure the circulation of air in the air-chamber. A thermometer passes from the top into the interior of the sterilizer so that one may read off the temperature that is being attained. The sterilizer should be placed so that there will be no danger of its setting fire to inflammable articles, as the heat may occasionally become very intense. It is well, if possible, to have it fastened to a brick wall.

Boiling.—Boiling is an efficient method of sterilization. It is often used for instruments. In laboratory work steam is generally substituted for it.

Steam Sterilization.—Steam sterilization is the most generally used of all forms of sterilization and is the most effective. It is employed for perishable bodies which would be injured by dry air sterilization or by chemical germicides; for example, it is used for surgical instruments and for culture-media; in laboratory work, especially for culture-media. It has been found that there are some forms of bacteria which, in the resting or spore stage, can resist even the action of steam for several hours. Such prolonged exposure to steam would be very injurious to culture-media, which are more or less unstable organic substances. What is called the *fractional* or *intermittent* method of sterilization is used for such materials. By that plan the medium is sterilized with steam for fifteen minutes on each of three consecutive days. The object of intermittent sterilization as explained by Tyndall, who proposed it, is this: The culture-medium may be supposed to contain fully developed bacteria, and also bacteria in the spore or resting stage. The first sterilization of fifteen minutes will probably be sufficient to destroy all the fully developed bacteria; during the twenty-four hours between the first and second sterilization, all of the spores which have survived the first sterilization may be expected to have become fully developed into bacteria which can be destroyed by the second sterilization; the third sterilization is directed against any spore forms which may possibly have survived the second sterilization.

Although the spore forms which are so extremely resistant are mostly non-pathogenic, as for example the bacilli of hay and potato, they nevertheless are capable of ruining the culture-media with which one works.

The form of sterilizer most widely used in the United States is that which is known as the Arnold Steam Sterilizer.

The Arnold sterilizer consists of a cylinder of tin or copper with a cover, which is covered with a cylindrical outer cover or hood. The inner cylinder has an opening in the bottom through which steam may enter, the steam coming from a small chamber underneath with a copper bottom to which the flame is applied. The peculiarity of this form of sterilizer consists in the fact that the steam which escapes from the sterilizing chamber will be condensed beneath the outer cover or hood and will fall back upon the pan over the chamber in which the steam is generated. The bottom of this pan is perforated with three

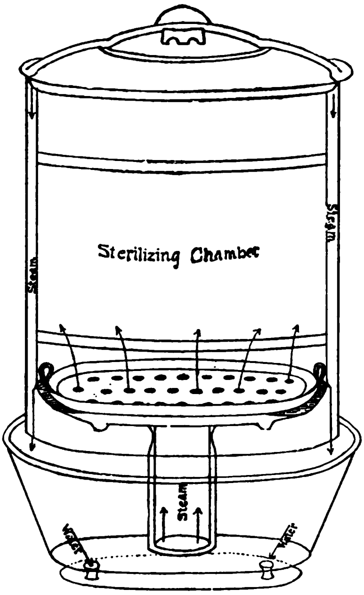


FIG. 13.—Diagram of the Arnold Steam Sterilizer.

small holes which allow the water of condensation to return into the chamber where the steam is generated. The sterilizer will, therefore, to a certain extent, supply itself with water, although not by any means perfectly. It is, however, less likely to boil dry than other forms of sterilizers, and it has the advantage of being reasonably cheap and quite effective. The space enclosed by the hood also serves as a steam-jacket and helps to overcome fluctuations in temperature. A great improvement upon the ordinary Arnold sterilizer is the modification of it devised by the Massachusetts Board of Health.

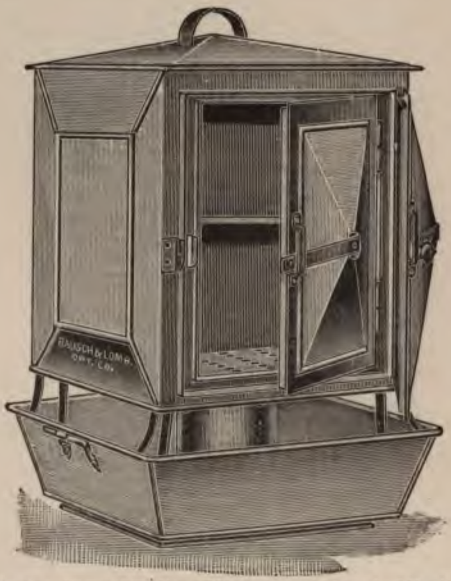


FIG. 14.—Steam Sterilizer, Massachusetts Board of Health.

In the use of this, or any form of steam sterilizer, the time when sterilization is supposed to begin must be counted as that when boiling is brisk and it is evident that the sterilizing chamber is filled with hot steam ; or, what is better, when the thermometer registers 100° C., if the sterilizer is provided with a thermometer.

The sterilizer invented by Koch is still largely in use, in fact almost universally, except in the United States. This sterilizer is a tall cylindrical tin vessel covered with asbestos or felt. The lower portion is filled with water; on the side is a water-gauge indicating the height of the water, in order that one may observe when there is danger of the sterilizer boiling dry. Over the top there is a tight-fitting cover. The steam is generated by a Bunsen burner standing underneath. A perforated shelf placed some distance above the surface of the water is for the reception of the tubes and flasks that are to be sterilized.

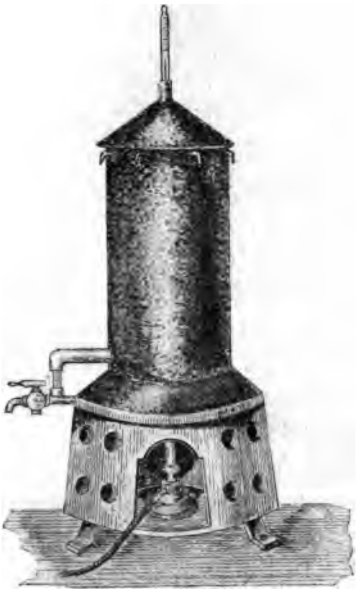


FIG. 15.—Koch Steam Sterilizer.

The sterilization of blood-serum sometimes has to be performed in a specially devised sterilizer, when a clear medium which has not been coagulated is desired. In this case the serum is heated for an hour on each of six consecutive days to a temperature of only 68° or 70° C.

Pasteurization.—The name pasteurization has been applied to the partial sterilization of substances at a comparatively low temperature. It is used particularly for milk. The temperature used is sufficient to destroy all ordinary pathogenic bacteria; for example, the bacilli of tuberculosis and typhoid fever. Furthermore, the great majority of the saprophytic bacteria are destroyed, and milk which has been pasteurized will remain unchanged for several days, if kept cool. Its application is principally in the feeding of infants when ordinary milk has been found to produce undesirable results. Freeman has invented a pail of special form for the pasteurization of milk in bottles. This pail is filled with hot water and the bottles are placed in it; it has been found to keep up a temperature of about 75° C.

The Autoclave.—The autoclave is an instrument designed for sterilization by steam under pressure. It is widely used in France, and to a less extent elsewhere, although an extremely convenient form of apparatus for certain purposes. Steam under pressure, or superheated steam, is much more destructive to germs than steam at the ordinary atmospheric pressure. In the autoclave steam is generated at a pressure of from one and one-half to two atmospheres; that is to say, 115° to 120° C. At such temperatures culture-media may be sufficiently sterilized in the autoclave in fifteen minutes, and in a single sterilization (Fig. 16). The autoclave consists of a metal cylinder with a movable top, which is fastened down tightly during sterilization. It is furnished with a thermometer, a pressure-gauge, and a safety-valve which allows the steam to escape if too high a pressure is attained. Heat is furnished by a gas-burner underneath. The lower part of the cylinder contains water. The objects to be sterilized are supported above this water on the perforated bottom.

It is necessary to follow certain precautions in the use of the autoclave, especially during cooling. The apparatus must not be opened while the steam contained within it is still under pressure, as there may be a sudden evolution of steam upon the removal of the pressure which may blow the media out of their tubes and flasks. The apparatus must, therefore, be kept closed until the gauge shows that the atmospheric pressure is as great as the pressure within, or, what is equivalent, until the temperature has fallen to 100° C. Gelatin, especially, may be damaged by sterilization with the autoclave, and should be left in it a shorter time—five minutes.

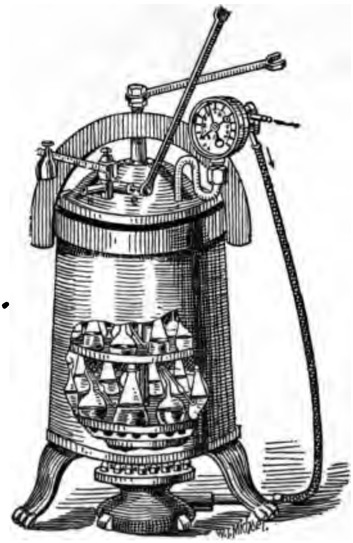


FIG. 16.—Autoclave.

Sterilization by Filtration.—Ordinary filters are useless for this purpose, but the tube of unglazed porcelain devised by Pasteur and Chamberland is effective when properly employed. This tube is widely used in filtering water for domestic purposes, and will be spoken of in connection with the chapter on water. Similar tubes are employed for the filtration of certain organic nutrient media whose ingredients would be damaged by sterilization with heat, chiefly extracts of organs, such as the thymus gland. The "toxins" of bacteria may be obtained by filtration of fluid-cultures through such tubes, which remove the bacteria (Fig. 17). The cultures usually filter very slowly, and filtration will have to be assisted by some form of vacuum-pump; usually the filter-pump, which is used in connection with a stream of running water, is employed. The porcelain tubes, the flasks and all parts of the apparatus must, of course, be sterilized by heat before and after using.

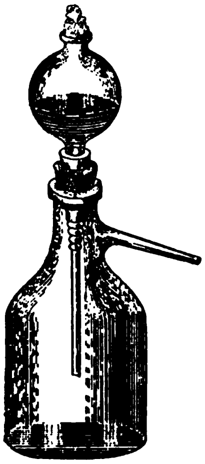


FIG. 17.—Kitasato Filter.

CHAPTER III.

CULTURE-MEDIA.

CULTURE-MEDIA are substances in which bacteria are artificially cultivated. The number of such substances is very large, different materials being suited to different purposes and to different kinds of bacteria. The most important ones are nutrient bouillon or beef-tea, nutrient gelatin, and nutrient agar agar. The two last are of the same composition as bouillon, and also have a jelly-like consistency, owing to the addition of a gelatinizing substance.

NUTRIENT BOUILLON.

Beef-extract (such as Liebig's),	3 grams.
Peptone, pure (Witte's),	10 grams.
Sodium chloride (common salt),	5 grams.
Water,	1 liter.

The solid ingredients are dissolved in water, and the mixture is boiled for a few minutes. It is made neutral or very faintly alkaline by the addition of a solution of sodium hydrate, drop by drop, the reaction being tested at intervals with litmus-paper. The bouillon may now be filtered through filter-paper. The filter-paper should be folded and creased as is done by pharmacists; it is usually placed in a glass funnel, and should be moistened with water before using. After filtration the medium is to be placed in properly plugged tubes or flasks, and is to be sterilized in the steam sterilizer for fifteen minutes or longer on each of three consecutive days. When precipitates form, they are usually caused by a too alkaline reaction. That may be corrected by the addition of a little weak hydrochloric acid, drop by drop, testing frequently with litmus-paper.

A more accurate way of obtaining the proper reaction is Schultz's method. Take of the bouillon 10 c.c.; add a few drops of phenolphthalein (alcoholic solution $\frac{1}{3}$ per cent.), with a burette; add, drop by drop, a solution of caustic soda 0.4 per cent. until a faint red color appears, which indicates the beginning of the alkaline reaction. This procedure is followed with three samples. The amount of soda solution required in each case is noted and the average taken. If now, on the average, for each 10 c.c. of bouillon 1 c.c. of soda solution needs to be added, for 1000 c.c. of bouillon 100 c.c. of the soda solution must be added; only, instead of adding a weak soda solution, one-tenth as much is taken of a solution ten times as strong.

The original method of making bouillon was to use, instead of beef extract, 500 grams (one pound) of lean beef chopped fine, which was placed in one liter of water and kept on ice for twenty-four hours. It was strained and a liter of fluid obtained. The other ingredients were then added and the medium thoroughly cooked to coagulate the albumen in it, after which it was treated as the bouillon above described.

Bouillon may be modified by the addition to it of other substances, the most important of which is sugar, either dextrose,* saccharose or lactose.

NUTRIENT GELATIN.

Beef-extract, ...	3 grams.
Peptone, ...	10 grams.
Sodium chloride, ...	5 grams.
Gelatin (best gold label), ...	100 grams.
Water, ...	1 liter.

---

* Dextrose is the principal ingredient of commercial grape-sugar and should be obtained in a pure condition. One per cent. of either of these sugars *may* be added.

Dissolve the ingredients in the water, stirring actively to prevent burning at the bottom. It is best to conduct the operations in granite- or enamel-ware vessels over a large Bunsen or rose-burner. Neutralize with sodium hydrate solution (see page 51). The reaction at the beginning will usually be found to be quite acid. Allow the mixture to cool until below 60° C., and add the whole of an egg which has been beaten up with a little water; stir it in thoroughly. Heat the mixture to the boiling-point; stir at the bottom to prevent burning and at the same time avoid as far as possible breaking the coagulum of egg-albumen which forms at the surface. Boil for ten minutes. Filter while hot. The filtration may be done through folded filter-paper which has been moistened. It is well to fasten a piece of coarse cheese-cloth over the top of the funnel to catch the large particles of coagulated albumen. Place in suitable tubes or flasks plugged with cotton, and sterilize in a steam sterilizer for fifteen minutes on each of three consecutive days. Gelatin is injured by too prolonged boiling and loses its solidifying qualities. Instead of the beef extract, fresh, chopped beef may be used as with bouillon. Neutralization may be with litmus-paper or by titration according to Schultz's method. Instead of filter-paper, some prefer to use several layers of absorbent cotton placed inside of the moistened glass funnel, the top of which is covered with coarse cheese-cloth. This expedient answers very well.

If the product appears cloudy after it has been sterilized, it may be that the egg-albumen was incompletely coagulated in the first place or that the reaction has been made too alkaline. In any case it will be desirable to melt it and filter a second time, correcting the reaction if necessary. It may be well to stir in another egg to entangle the opaque particles; then to boil a second time and filter.

The medium is sometimes modified by adding to it other substances, as sugar, glycerin, etc. The solidifying property of the gelatin must be carefully guarded, and too much boiling is to be avoided. Certain bacteria, it will be found, have the property of causing gelatin to become fluid. Gelatin melts at about 25° C. and solidifies at about 10° C. It cannot be used in the incubator, where it would liquefy at the temperature of 38° C. In hot weather it may be necessary to use 150 grams of dry gelatin to the liter. Nutrient gelatin is usually spoken of simply as "gelatin."

Nutrient Agar-agar.—*Agar-agar* is a kind of vegetable gelatin which comes from the southern and eastern coast of Asia. It melts with much greater difficulty than gelatin. The medium is not quite transparent. The finished medium is commonly called "agar."

Beef-extract,	3 grams.
Peptone,	10 grams.
Sodium chloride,	.5 grams.
Agar,	10 grams.
Water,	1 liter.

The dry agar, cut fine, is to be dissolved in water over a flame. It should be boiled for from one-half hour to two hours, skimming off the scum which forms on the surface from time to time. The beef-extract, peptone and sodium chloride are dissolved in a liter of water, boiled and neutralized. Add the agar now in solution in a small quantity of water. The reaction of the agar alone is faintly alkaline. Mix thoroughly; the bulk of the mixture is a little more than a liter, and should be reduced to a liter after the subsequent boiling. Cool to about 60° C.; stir in an egg, and boil thoroughly. Avoid breaking the coagulum of the egg which is designed to entangle the solid particles that make the medium cloudy; stir at the bottom, however, to prevent burning. Filter while hot, using filter-paper or absorbent cotton covered with cheese-cloth. The hot water funnel originally devised for the filtration of agar is not necessary. If filtration is slow, the funnel and flask may be placed inside of the steam sterilizer and kept heated during filtration. The medium is collected in suitable flasks or tubes plugged with cotton, and sterilized for fifteen minutes on each of three consecutive days. As agar is frequently used for smear-cultures where a slanted medium is desired, some of the tubes may be allowed to cool in a slanting position. For this purpose a form of wire basket with an inclined bottom is used by the writer (Fig. 18). It is not well to keep on hand many tubes which have been slanted, as the medium dries more rapidly. Agar is not liquefied by bacteria as is gelatin. Its solidifying qualities are impaired somewhat if the reaction be acid.

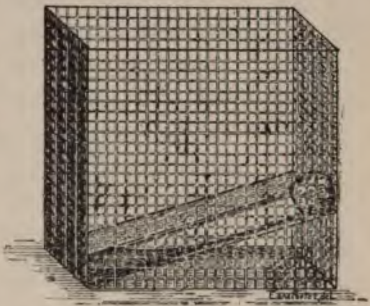


FIG. 18.—Wire Basket to hold Tubes of Culture-Media with a Slanted Surface.

Glycerin-agar is used extensively. It is agar, made as above directed, to which 5 per cent. of glycerin is added before sterilization. It is very useful in cultivating the bacilli of tuberculosis and of diphtheria.

Before sterilizing, 1 per cent. of either dextrose, lactose or saccharose may be added to agar. With media containing sugar, litmus forms a useful indicator of the production of acid. Enough tincture of litmus is used to give the medium a blue color before sterilization; the litmus is somewhat unstable and prone to change its color during sterilization.

Potato.—The potatoes are washed, a slice is removed from each end, and with an apple-corer or cork-borer a cylinder is cut out. This cylinder is divided diagonally into two pieces. The pieces are washed in running water for an hour or more. They are placed in test-tubes and are supported from the bottom on a piece of glass tubing about 1 to 2 c.m. in length (or on cotton, or in a specially devised form of tube with a constriction at the bottom). The tubes are plugged, and sterilized on three consecutive days as with other media. Sterilization, however, must be thorough on account of the danger of contamination with the extremely resistant spores of the potato bacillus. Potato is best when freshly prepared; it is likely to become dry and discolored with keeping. It is a very useful medium; certain growths on it, like those of the bacillus of typhoid fever or of glanders, and those of chromogenic bacteria, are very characteristic.

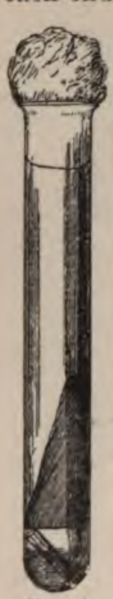


FIG. 19.—Tube Containing Potato.

Milk.—Milk fresh as possible is placed in a covered jar on the ice for twenty-four hours. At the end of that time the middle portion is removed by means of a siphon. The upper and lower layers must not be taken; the upper part contains cream, and the lower part particles of dirt, both of which are to be avoided. About 10 c.c. are to be run into each test-tube. The tube is plugged with cotton, and sterilized on each of three consecutive days, for fifteen minutes or longer. When milk is contaminated with spores of the hay or potato bacillus it is sometimes very difficult to sterilize, a fact of much importance in connection with the feeding of children.

The coagulation of milk, which is accomplished by certain bacteria, is a very valuable differential point. A little litmus tincture may be added to the tubes of milk before sterilizing, until they acquire a blue color, to indicate whether or not acids are formed by the bacteria growing in the medium.

Blood-serum.—The blood of the ox or cow may be obtained easily at the abattoir. It should be collected in a clean jar. When it has coagulated, the clot should be separated from the sides of the jar with a glass rod. It may be left on the ice for from twenty-four to forty-eight hours. At the end of that time the serum will have separated from the clot and may be drawn off with a siphon into tubes. These tubes are sterilized for the first time in a slanting position, as the first sterilization coagulates the serum. (The coagulation may be done advantageously, as advised by Councilman and Mallory, in the hot-air sterilizer at a temperature below the boiling-point. A wire basket with an inclined bottom will be found convenient for coagulating serum tubes in the slanting position (see Fig. 18). After coagulation, sterilize in the steam sterilizer as usual. This serum makes an opaque medium of a cream color. Blood-serum may be sterilized in the special form of sterilizer devised for it. A clear blood-serum is to be obtained by sterilization at a temperature of 70° C. for one hour, on each of five days. At the end of that time the serum is heated to 75° or 80° C., in an inclined position, to solidify it. Opaque, coagulated blood-serum has most of the advantages of the clear medium. Blood-serum may be secured from small animals by collecting blood directly from the vessels, using very great care to obtain the blood in a sterile condition; and the serum may be separated and stored in a fluid state. Human blood-serum is sometimes obtained from the placental blood, sometimes from serous pleural transudates or from hydrocele fluid. The preservation of blood-serum is sometimes accomplished with chloroform, of which 1 per cent. is to be added to the medium; in this manner the serum may be preserved for a long time. It may be divided into tubes, solidified and sterilized as required; the chloroform will be driven off by the heat, owing to its volatility. Blood-serum media which are sterilized at a low temperature should be tested for twenty-four hours in the incubator to prove that sterilization has been effective; if not, development of the contaminating bacteria will of course take place and be visible to the eye.

DUNHAM'S PEPTONE SOLUTION.

Peptone,	10 grams.
Sodium chloride,	5 grams.
Water,	1 liter.

Boil, filter, sterilize in the usual manner.

Dunham's solution is valuable to test the development of indol by bacteria. The development of acids may be detected after the addition of 2 per cent. of rosolic acid solution (.5 per cent. solution in alcohol); alkaline solutions give a clear rose-color which disappears in the presence of acids.

It will be impossible to do more than merely mention some of the most important of the other culture-media.

Löffler's blood-serum consists of one part of bouillon containing 1 per cent. of glucose, and three parts of blood-serum. It is used largely for the cultivation of the bacillus of diphtheria.

Blood-serum-agar is a medium made with considerable difficulty, but very valuable for the cultivation of the gonococcus. The placental blood-serum, or the pleuritic serum or hydrocele fluid, is mixed with nutrient agar in the fluid condition and must be divided into tubes before solidification. Solidify in a slanting position; subsequently sterilize so as not to coagulate the albumen of the blood-serum. One expedient has been also to smear a little blood over the surface of a tube of nutrient agar—*blood-agar*—used for cultivating the bacillus of influenza. Marmorek's blood-serum is supposed to assist in maintaining the very evanescent virulence of the streptococci; bouillon is mixed with human blood-serum, ass's serum or horse's serum.

Guarnieri's medium consists of a mixture of gelatin and agar.

Media containing *fat* were employed by Sommaruga to test the ability of bacteria to decompose fats. Clarified beef-suet or olive-oil in the proportion of one or two per cent. is added to gelatin or agar. The fat must be mixed with the melted medium; it is to be shaken and then rapidly cooled in a freezing-mixture after the last sterilization.

Fresh eggs in their shells may be used without other preparation than washing the surface thoroughly with bichloride of mercury solution; or after sterilization by steam, which of course coagulates the albumen. The egg is easily inoculated through a small opening made with a heated needle, which may be closed afterward with collodion. Hueppe recommended eggs closed in this manner for the cultivation of anaërobic bacteria. Egg-albumen has been used as a constituent of various media.

Elsner's medium for the detection of the bacillus of typhoid fever is an acid, 10 per cent. gelatin, made up with potato-water. Potato-water is the expressed juice of potatoes which have been cleaned, peeled and grated. Allow the juice to settle; filter while cool, boil and again filter. The medium is made up without peptone and sodium chloride; add 1 per cent of iodide of potassium.

Bread-paste (finely-divided dry bread, mixed with water and sterilized) is used for the cultivation of moulds. Sabouraud recommends the following for the cultivation of the tricophyton fungus:

Peptone,	5 grams.
Maltose,	3.8 grams.
Agar,	1.3 grams.
Water,	100 c.c.

Test-tubes.—Bacteria are generally cultivated in test-tubes. A convenient size is one ⅝ of an inch in diameter and 5 inches in length. The tubes should be of a heavier glass than in those used for ordinary chemical work. The New York Board of Health, and some others, use a tube three inches in length without a flange for the cultivation of the diphtheria bacillus on Löffler's blood-serum mixture. Test-tubes should be thoroughly cleaned with a swab before using; they should be boiled with washing-soda, rinsed, filled with hydrochloric acid solution, rinsed, and inverted to drain away the fluid.

Plugs of raw cotton or cotton batting are employed as stoppers. The plug should fit smoothly; creases and cracks around the edges are to be avoided. The plug should be tight enough to sustain the weight of the tube when held by the plug. These plugs prevent bacteria from entering or leaving the tubes.

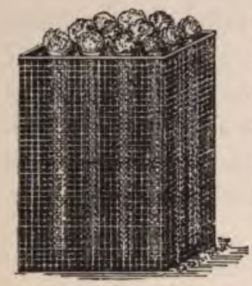


FIG. 20.—Wire Basket for Test-tubes.

Sterilization of Test-tubes.—The tubes are to be sterilized in a hot-air sterilizer for one hour, at a temperature of 150° C. or higher. The cotton should acquire a light brown color but should not be burned. If the plugs touch the sides of the sterilizer or lie against the bottom they may be scorched.

The necessity for sterilization of the tubes before filling them with the medium has been questioned, and it is probably unnecessary as far as the preservation of the culture-medium is concerned, but it will be found that the cotton plugs fit much better after sterilization with dry heat. During this and subsequent sterilizations the tubes are held in a wire basket.

Filling of the Tubes.—A special funnel closed with a stop-cock for filling tubes with liquefied media is often recommended. They may readily be filled with an ordinary funnel of small size. During the filling, the neck of the test-tube where it comes in contact with the cotton *must not be wet* with the medium. Ordinarily about 10 c.c. are placed in a test-tube. For Esmarch's roll-tubes a somewhat smaller quantity is desirable.

The Sterilization of tubes containing culture-media is always done by steam, and, as we have seen, in most cases is for fifteen minutes or longer on each of three consecutive days. It is to be remembered that the solidifying power of gelatin is impaired by too prolonged heating, while heating is of less importance with the other culture-media. The media which are sterilized at a low temperature (70° C.) should be tested for two days in the incubator to see whether sterilization has been effective. It is the universal experience in bacteriological laboratories that occasionally culture-media will become contaminated with extremely resistant spores which fail to be sterilized by the ordinary processes, an occurrence which causes great annoyance and calls for the exercise of much patience. Sometimes, also, moulds attach themselves to the plugs, especially if they are moist, and send their filaments down through the cotton; finally, having reached the lower edge of the cotton, their spores may fall upon the medium and ruin it.

CHAPTER IV.

THE CULTIVATION OF BACTERIA.

Inoculation of the Tubes.—The air of the laboratory should be as quiet as possible, to lessen the chances of contamination by bacteria clinging to particles of dust. Avoid working where there may be draughts or gusts of air. Given any material containing bacteria, for example a pure culture of some well-known species, a very minute portion is to be introduced into a tube containing the sterile culture-medium. The introduction is effected with a straight platinum wire, or with a platinum wire loop. The platinum is to be heated red-hot before using, and then allowed to cool. It is also to be heated red-hot after using. The plug of the test-tube is to be withdrawn, twisting it slightly, taking it between the third and fourth fingers of the left hand, with the part that projects into the tube pointing toward the back of the hand. It must not be allowed to touch any object while the inoculation is going on. If any of the cotton adheres to the neck of the tube, pass the neck through the flame of the Bunsen burner, or if necessary pull the cotton away with sterilized forceps. The tube is to be held in the left hand between the thumb and forefinger, the tube resting upon the palm, and the neck of the tube pointing upward and to the right. When two tubes are being used at the same time, as is often necessary, they are placed side by side between the thumb and forefinger of the left hand. The two plugs are held between the second and third and the third and fourth fingers of the left hand, respectively. The wire may now be passed into the first tube, which we will suppose to hold some material containing bacteria, and a little of this material may be removed on the tip of the wire from the first tube to the second. When the needle is introduced into or removed from either tube it should not touch the side of the tube at any point, and should only come in contact with the region desired. After inoculation of the second tube has been effected the wire is to be heated to a red heat in the flame, the necks of the tubes are to be passed through the flame, and the plugs are to be returned to their respective tubes. When the wet wire is to be sterilized in the flame it should be approached to the flame gradually, so as to dry the material on it before burning it, in order to avoid "sputtering" (see page 21). It is well from the start to train one's self to sterilize the platinum wire every time it is taken from the table and before it is laid down again. The platinum wire loop may be used in the same manner as the straight wire, especially when a substance containing a small number of bacteria is being handled.

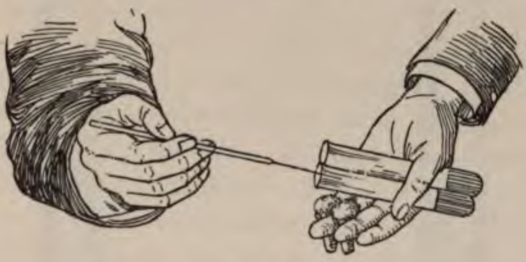


FIG. 21.—Manner of Holding Tubes.

When a tube of gelatin is to be inoculated the wire is usually introduced into the medium vertically, "stab-culture;" when a medium with a slanted surface is employed, as agar, potato or blood-serum, the needle should lightly streak the surface, "smear-culture" (Figs. 22 and 23).

The safety and success of this method of inoculation depend upon a principle which has been established by long and repeated observation, namely, that bacteria do not of themselves leave a moist surface. They should not, therefore, rise from the surface of the moist culture-medium, nor drop from the needle during its transit, if proper care be exercised. They may be thrown into the air if the needle be allowed to sputter in the flame.

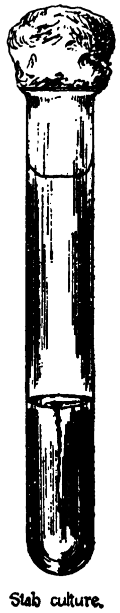

Stab culture.

FIG. 22.

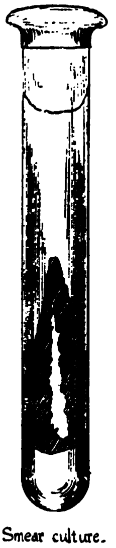

Smear culture.

FIG. 23.—This tube shows the rubber cap used to prevent drying.

To maintain their vitality bacteria need to be transplanted from one tube to another occasionally; the time varies greatly with different species. Many bacteria grow on culture-media with difficulty at the first inoculation, but having become accustomed to their artificial surroundings, as it were, they may be propagated easily afterward; this is especially true of the bacillus tuberculosis.

Some bacteria flourish better on one culture-medium than another. The bacillus tuberculosis grows best on blood-serum and glycerin-agar; the bacillus of diphtheria grows best on Löffler's blood-serum.

The virulence of most pathogenic bacteria becomes diminished after prolonged cultivation upon media. Sometimes the virulence is lost very quickly, for example, with the streptococcus pyogenes and micrococcus lanceolatus of pneumonia.

Incubators.—Many bacteria flourish best at a temperature about that of the human body, 38° C. Some species will grow only at this temperature. The pathogenic bacteria in particular, for the most part, thrive best at a point near the body temperature.

The incubator is a box made of copper, having double walls, the space between the two being filled with water. The outer surface is covered with some non-conductor of heat, such as felt or asbestos. At one side is a door, which is also double. The inner door is of glass, the outer door is of copper covered with asbestos. At one side is a gauge which indicates the level at which the water stands in the water-jacket. The roof is perforated with several holes, some of which permit the circulation of the air in the air-chamber inside the box; some of them enter the water-jacket. A thermometer passes through one of these holes into the interior of the air-chamber and another into the water standing in the water-jacket. A gas-regulator passes through another hole, and is immersed in the water standing in the water-jacket. There are various forms of gas-regulators more or less complicated. In general they consist usually of a tube containing mercury; into this tube are two openings, one for the entrance and the other for the exit of gas. The gas enters through a small tube, which is cut off diagonally at the bottom, and which projects into the surface of the mercury. Heating the water in the water-jacket causes expansion of the mercury, which rises, and, little by little, cuts off the inflow of gas through this tube. The flow is never completely cut off, as there is a capillary opening in the tube considerably above any point to which the mercury could possibly rise, which will always allow the flow of a small quantity of gas (Fig. 26, *b*). This diagram also shows a modification of the simple form of regulator, in the shape of a partition which divides off a lower

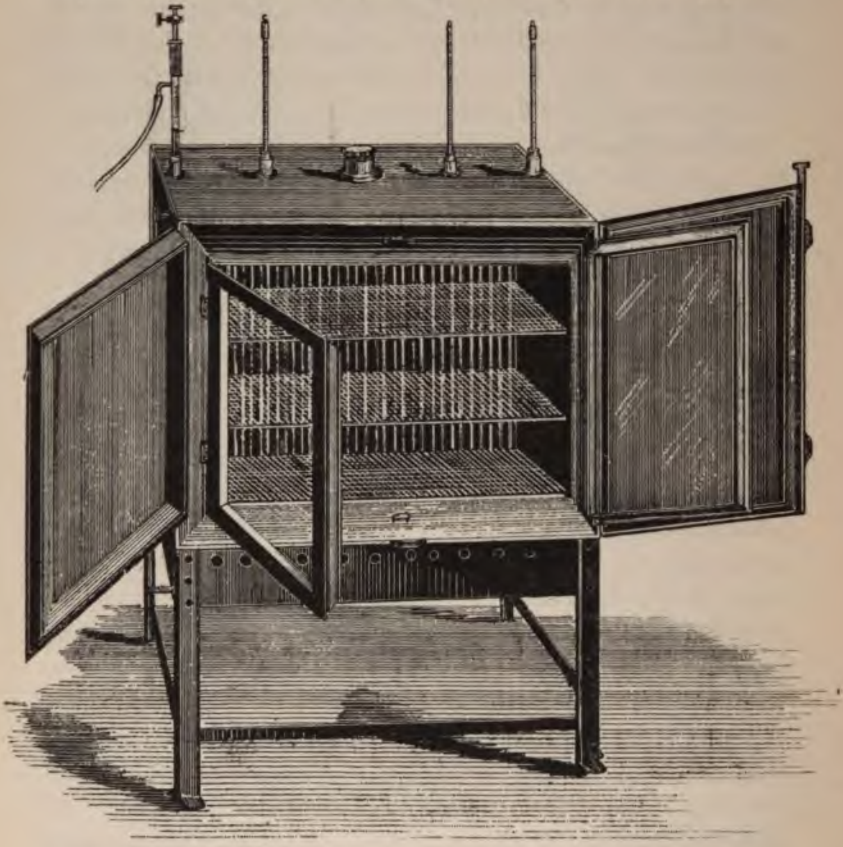


FIG. 24.—Incubator. (The gas-burner and the supply-tube running to it from the gas-regulator have been omitted ; see Figs. 25, 26, and 27.)

chamber, which contains mercury and is connected with the upper part by a glass tube. The purpose is to make use of the elastic properties of some volatile fluid, like ether, which floats on the surface of the mercury at *a*. The gas coming from the gas-regulator passes to a Bunsen burner, which stands underneath the incubator. This burner should have some kind of an automatic device for cutting off the flow of gas in case it becomes accidentally extinguished by a sudden draught of air or from any other cause. The automatic burner invented by Koch is an ingenious, simple and effective device. A bar of metal stands above the flame; by its expansion, through a system of levers, it supports a weight; the weight controls a gas-cock. While the flame is burning the expansion of the metal holds the weight horizontally; if the flame becomes extinguished, the

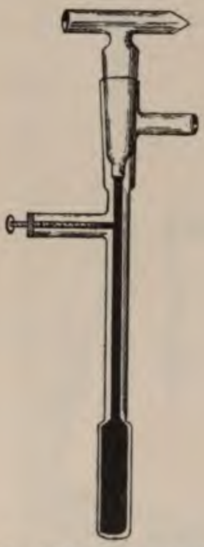


FIG. 25.—Reichert's Gas-regulator.

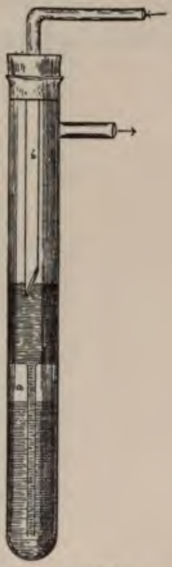


FIG. 26.—Mercurial Gas-regulator. *a*. Chamber containing volatile hydrocarbon. *b*. Capillary opening.

metal contracts, the weight falls, and cuts off the flow of gas. Some inconvenience will arise from irregularities in the flow of gas from the main supply-pipe. Any incubator will vary a little from such causes. In the experience of the writer, natural gas is of such variable pressure as to be entirely useless. Fluctuations of the temperature within the incubator depend very largely upon the external temperature. Therefore the incubator should, as far as is practicable, be protected from sudden draughts of cold air and should be kept in a room having as equable a temperature as possible.

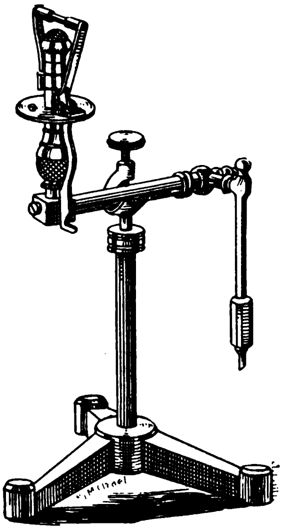


FIG. 27.—Koch Automatic Gas-burner.

Culture-tubes which are being kept in the incubator are likely to become dry if their stay is prolonged. In such cases they should be covered with rubber caps, sealing-wax, paraffin, or some other device to prevent evaporation. If rubber caps are used, they should be left in 1–1000 bichloride of mercury for an hour, and the cotton plugs should be singed in the flame, before putting them on (Fig. 23).

Cultivation of Anaërobic Bacteria.

The cultivation of anaërobic bacteria is done best in a medium containing 1 to 2 per cent. of dextrose. The tube should contain a large quantity of the culture-medium. Just before using, the medium should be melted and boiled for a few minutes. Inoculate the tube after cooling, but while the medium is fluid.

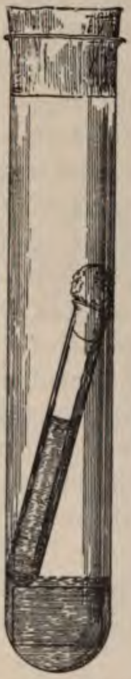


Fig. 28.—Arrangement of Tubes for Cultivation of Anaërobes by Buchner's Method.

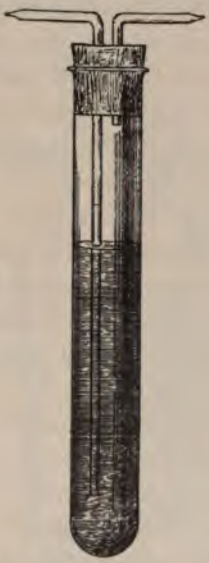


Fig. 29.—Cultivation of Anaërobes by Fränkel's Method.

Buchner's method for the cultivation of anaërobes: Into a tube or bottle which can be tightly stoppered, pour 10 c.c. of a 10 per cent. solution of potassium hydrate and add a gram of pyrogallic acid. The culture-tube is placed inside of the larger tube or bottle, supported above the bottom, and the large tube is to be tightly closed. The mixture of pyrogallic acid and potassium hydrate possesses the property of absorbing oxygen.

CULTIVATION OF ANAËROBIC BACTERIA UNDER HYDROGEN.

Method of Fränkel: A test-tube containing a large amount of the liquefied culture-medium is closed with a sterilized rubber stopper, through which pass two sterilized glass tubes, bent above the stopper at a right angle. One of these tubes is cut off just underneath the stopper, and the other is long enough to project nearly to the bottom of the culture-tube. The horizontal projecting parts are drawn to a small caliber at some point, although not quite closed, to facilitate sealing later on. Through the longer of these tubes hydrogen gas is passed until the atmosphere inside of the culture-tube is pure hydrogen, entirely free from mixture with air. The horizontal parts of the small glass tubes projecting from the stopper are then sealed in the flame at the places where they were previously drawn out to a small caliber, and the tubes are thus closed (Fig. 29).

The stopper should be surrounded with melted paraffin. A tube prepared according to this plan may, if desired, be converted into an Esmarch roll-tube. The hydrogen is generated according to the common method with *pure* zinc and *pure* sulphuric acid, 25 to 30 per cent. The precautions advised by chemists for the generation of hydrogen must be carefully followed, because when hydrogen mixed with oxygen or air is ignited a violent and disastrous explosion may occur.

The well-known Kipp's generator may be used. First let the reservoir fill with hydrogen; then allow its contents to escape. This should be repeated, after which some of the hydrogen may be collected in an inverted test-tube under water. When this sample is ignited, it should burn without any explosion; otherwise the hydrogen is not yet ready to use. The hydrogen should bubble through the medium five minutes or more.

The inconvenience of sealing the tubes in the flame, as has to be done in Fränkel's and other methods for cultivation under hydrogen, is obviated in Novy's APPARATUS. The tubes or plates are placed in jars through which hydrogen may be conducted. The stopper, having been smeared previously with a soft wax, is sealed by giving it one-fourth of a turn.

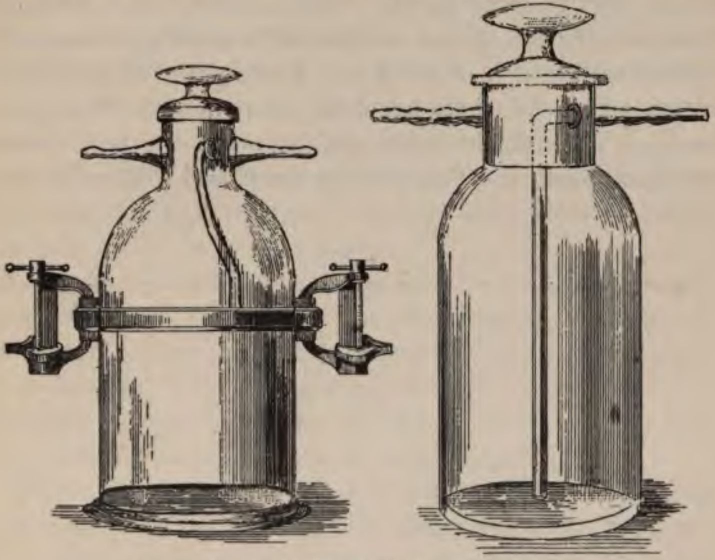


FIG. 30.—Novy's Jars for the Cultivation of Anaërobes.

There have been various other kinds of apparatus, usually complicated and expensive, devised for the growth of plate-cultures under hydrogen. Other expedients for the cultivation of anaërobic bacteria are less effective. In cases where a very deep stab-culture is made in gelatin or agar, where the growth appears in the lower part of the tube by preference, it is supposed to be anaërobic. Koch covered part of the surface of a gelatin plate with a bit of sterilized mica or a cover-glass; bacteria which grew beneath this plate were considered to be anaërobic. Another method was to cover the surface of the gelatin in the culture-tube with sterilized oil. Esmarch advises making roll-tubes, and after cooling them to fill them with a liquefied gelatin cooled down to near the point of solidification. Hueppe made use of eggs in their shells. The eggshell was carefully cleaned, sterilized with a solution of bichloride of mercury, washed with sterilized water and wiped dry with sterilized cotton. The end of the eggshell was punctured with a hot needle. Through the opening thus made the inoculation was accomplished. The opening was closed with collodion.

CHAPTER V.

CULTIVATION OF BACTERIA, CONTINUED.

Isolation of Bacteria.—In order to study any kind of bacteria it is necessary to have the particular species separated from other sorts with which it may be mixed. The earlier bacteriologists endeavored to separate bacteria of different sorts by successive transplantations through a series of tubes. The procedure now generally used for this purpose is the so-called plate-method of Koch. The great progress which bacteriology has made during the last twenty years is largely owing to this invention.

Pathogenic bacteria may sometimes be isolated through inoculations into animals. Thus an animal may be inoculated with sputum containing tubercle bacilli mixed with other bacteria. The animal may die of tuberculosis, and its tissues may contain tubercle bacilli in pure culture, the other bacteria having produced no important effect.

Still another method which is occasionally useful is to subject the mixture of bacteria to steam for a few minutes. If it contains very resistant spores, like those of the tetanus bacillus or hay bacillus, they may be expected to survive, and may perhaps be propagated in pure culture, everything else having been killed by the steam.

Plate-cultures.—It is impossible in most cases to distinguish between bacteria of different varieties by microscopical examination alone. Bacteria of widely different species and quite unlike one another in their properties may present similar appearances under the microscope. The differences which they exhibit are usually apparent when they are grown in culture-media. The growth, called a *colony*, which develops from the multiplication of a single bacterium, is in many cases quite characteristic for the species. By the plate-method, the individual bacteria in a mixture are separated from one another by dilution. They are fixed in place by the use of a solid medium. They are allowed to grow, and from each individual there forms a colony. It is usually possible to distinguish between *colonies* arising from different species when it was not possible to distinguish between *the individual bacteria* of these species. A convenient illustration has been suggested by Abbott. A number of seeds of different sorts may appear very much alike, and considerable difficulty may be found in distinguishing one from the other with the eye. Let them be sown, however, and let plants develop from them, and these plants will easily be distinguished from one another.*

Method of Making Plate-cultures.—Melt *three* tubes of gelatin or agar. (There is some difficulty in keeping agar in a fluid state while dilutions are being made. It is best to have some form of water-bath with a thermometer for the purpose.) Let the liquefied tubes cool to 40° C. Take a small portion of the material to be examined—pus, for example—and introduce it with a sterilized platinum wire or loop into one of the tubes. Stir it in carefully. Remove the needle, sterilize it, and replace the plug. Mix the material introduced thoroughly with the liquefied culture-medium, taking care not to wet the plug. Now remove the plug again, and, having sterilized the platinum wire, insert it into the liquefied medium. Carry three loopfuls in succession from this tube, which is No. 1, into tube No. 2;

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* It must be understood that *no close comparison can be drawn between higher plants*, which simply complete the development of parts potentially present in the seed, *and colonies of bacteria*, which are aggregates of individuals, the progeny of one individual of the same kind.

sterilize the needle; replace the plugs; mix thoroughly, without wetting the plug. Carry three loopfuls from tube No. 2 into tube No. 3 in the same manner. The original material will obviously be diluted in tube No. 1, more in tube No. 2, and still more in tube No. 3. The most convenient form of plate is that known as a Petri dish, a small glass dish about 8 cm. in diameter and 1.5 cm. in height, provided with a cover which is a little larger but of the same form. This dish should be cleaned and sterilized for an hour in a hot-air sterilizer at 150° C. or higher. When it is cool it may be used.

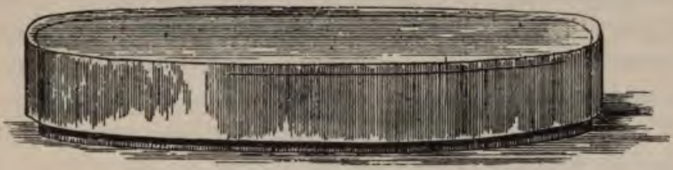

FIG. 31.—Petri Dish.

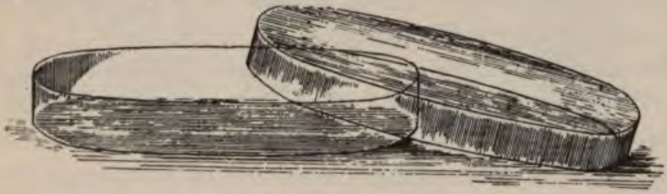

FIG. 32.—Petri Dish.

Such dishes having previously been prepared, the contents of tube No. 1 are poured into one dish, and those of tube No. 2 into another, and those of tube No. 3 into a third. They are to be labeled Nos. 1, 2, and 3.* In pouring proceed as follows: remove the cover of the dish; let one end rest upon the table, the other upon the dish proper;

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* The labels should be moistened with the finger, which has been dipped in water. They should not be licked with the tongue. While working in the bacteriological laboratory it is best to make it a rule that no object is to be put in the mouth.

the outer surface of the cover should be up (Fig. 32). The dish should be uncovered as short a time as possible. Remove the plug of tube No. 1; heat the neck of the tube in the flame; allow it to cool, holding it in a nearly horizontal position. When it has cooled, pour the contents into the Petri dish and replace the cover of the dish. Tubes Nos. 2 and 3 are to be treated in the same manner. Burn the plugs, and fill the empty tubes with 5 per cent. solution of carbolic acid. They should be sterilized for an hour in the steam sterilizer (see page 44).

The culture-medium in the Petri dish will soon solidify. Colonies develop usually in from one to two days. In plate No. 1 they will be very numerous, in plate No. 2 less numerous, and in plate No. 3 still less numerous. Where the number is small the colonies will be widely separated and can readily be studied. They may be examined with a hand-lens, or the entire dish may be placed on the stage of the microscope and the colonies be inspected with the low power. The iris diaphragm should be partly closed and the concave mirror should be used.

Esmarch's Roll-tubes.—Use liquefied gelatin or agar. The dilutions in tubes 1, 2 and 3 are made as above. Tubes containing a rather small amount of the culture-medium are more convenient. A block of ice should be at hand, and, with a tube filled with hot water and lying horizontally, a hollow of the size of the test-tube should be melted on the upper surface of this ice. In this hollow place the tube of liquefied gelatin or agar; roll it rapidly with the hand, taking care that the culture-medium does not run toward the neck as far as the cotton plug. The medium is spread in a uniform manner around the inside of the tube, where it becomes solidified. Gelatin roll-tubes must be kept in a place so cool that there is no danger of their melting; in handling them they are to be held near the neck, so that the warmth of the hand may not melt the gelatin. Agar roll-tubes should be kept in a position a little inclined from the horizontal, with the neck up, for twenty-four hours, so that the agar may stick to the wall of the tube.

By the plate-method as originally devised by Koch, instead of using Petri dishes, the gelatin was poured upon a sterile plate of glass. This plate of glass was laid on another larger plate of glass, which formed a cover for a dish of ice-water, the whole being provided with a leveling apparatus. The plate was kept perfectly level until it had solidified, which took place rapidly on the cold surface. The glass plates were placed on little benches enclosed within a sterile chamber. The more convenient Petri dish has displaced the original glass plate to a large extent.

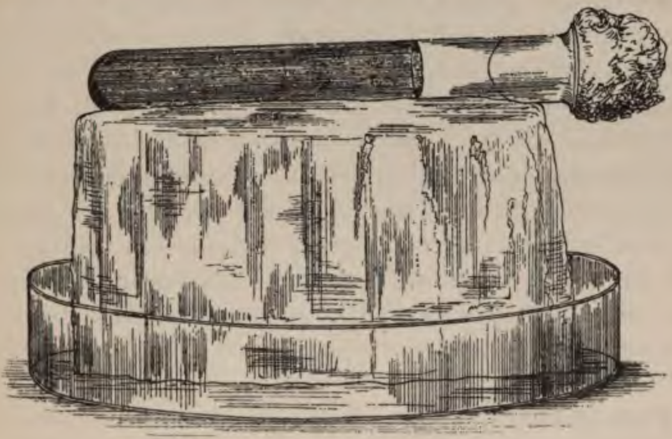


FIG. 33.—Manner of Making Esmarch Roll-tube.

The isolation of bacteria may sometimes be effected by drawing a platinum wire containing material to be examined rapidly over the surface of a Petri dish containing solid gelatin or agar; or over the surface of the slanted culture-medium in a test-tube; or by drawing it over the surface of the medium in one test-tube, then, without sterilizing, over the surface of another, perhaps over several in succession.

Appearance of the Colonies.—The colonies obtained in the Petri dishes or roll-tubes may be studied with a hand-lens or with a low-power microscope. In the latter case, use the concave mirror with the iris diaphragm partly closed. The colonies present various appearances. Some of them are white, some colored; some are quite transparent and others are opaque; some are round, some are irregular; some have a smooth surface, others appear granular, and others present a radial striation. Surface colonies often present different appearances from those occurring more deeply. Surface colonies are likely to be broad, flat and spreading. If the colony consists of bacteria which have the property of liquefying gelatin, a little funnel-shaped pit or depression forms at the site of the colony. The appearance of colonies may be of great assistance in determining the character of doubtful species. The appearance in gelatin plates of the colonies of the spirillum of Asiatic cholera, for instance, is one of the most characteristic manifestations of this organism.

Pure Cultures.—From these colonies pure cultures may be obtained by what is called "fishing." Select a colony from which cultures are to be made; touch it lightly with the tip of a sterilized platinum wire, taking great care not to touch the medium at any other point. Introduce the wire into a tube of gelatin. Sterilize the wire and plug the tube. In a similar manner, and from the same colony, inoculate tubes of agar, bouillon, milk, potato and blood-serum. At the same time it is well to make a smear preparation from the colony and to stain with one of the aniline dyes so as to determine the morphology of the bacteria. The growths which take place in the tubes should contain one and the same kind of bacteria. As seen under the microscope their bacteria should have the same general form and appearance as those seen in the colony from which they were derived. This will be the case, provided the colony has resulted from the development of a single bacterium or from several bacteria of the same kind. Occasionally, however, a colony will develop from several bacteria which may not all be alike. In that case a pure culture will not be obtained, and the process of plating may have to be repeated.

CHAPTER VI.

INOCULATION OF ANIMALS.

In the study of pathogenic bacteria, the inoculation of animals is frequently indispensable. The animals most often used are white mice, guinea-pigs, rabbits and pigeons. Larger animals are occasionally employed for special purposes. White mice may be kept in a glass jar covered with wire netting. They may be fed with moistened bread or oats. It is important to see that they receive drinking-water. During inoculation the mouse must be kept in position by some sort of mouse-holder, or may be held by an assistant, who takes the skin at the back of the neck between his fingers and at the same time holds the tail. The hair is cut off from the skin at the root of the tail. A small V-shaped opening in the skin is made with scissors, and a stiff sterilized platinum wire is passed into this opening, separating the skin from the muscles for some distance so as to make a pocket. Into this pocket the material is introduced by means of the platinum wire. The wound may be covered with collodion. The peritoneal

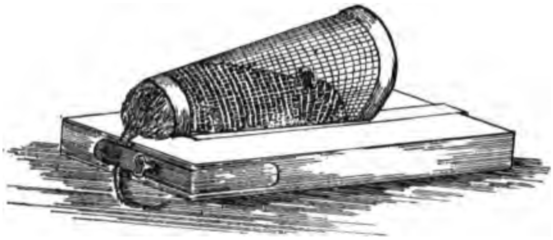


FIG. 34.—Mouse-holder.

cavity of the mouse may be inoculated with a fluid-culture introduced with a sterile hypodermic syringe.

Guinea-pigs and rabbits, after inoculation, are to be kept in cages of galvanized iron and wire-netting. The bottom may conveniently be made in the form of a movable pan which permits of the disinfection of the excreta. Rabbits and guinea-pigs may be fed with oats, carrots, cabbage, grass, and the like. Guinea-pigs and rabbits may be held by an assistant or tied by the legs upon a board. The hair over a small portion of the abdomen is cut away, and a short incision is made through the skin; a pocket is produced with a stiff wire, and the material introduced with a platinum wire. The wound may be covered with collodion. Sutures may be used if the wound is large. The peritoneal cavity may be inoculated with a previously sterilized hypodermic syringe; or an incision may be made which reaches to the peritoneal cavity, into which the desired substance may be introduced with a platinum wire, the incision being closed with sutures.

Intravenous inoculation is most commonly practiced upon rabbits. A small vein which is near the posterior margin of the ear of the rabbit is easily reached from the dorsal surface; the hypodermic needle is introduced directly into this vein. In making a hypodermic injection, the needle and syringe should of course be sterilized before and after each operation.

Autopsies upon animals should be held as soon as possible after death. During the interval the body should be kept on ice. The animal should be extended on its back upon a board. The legs may be fastened with pins or tacks. After beginning the autopsy the hand should not touch the animal. Knives, scissors and forceps should be sterilized in the flame after each manipulation. Be ready to make smear preparations on cover-glasses, and to inoculate tubes of gelatin, agar and other media as desired. Make an incision, passing through the skin from the sternum to the pubis along the thorax and abdomen, and diagonal incisions extending down the fore and hind legs. Dissect away the skin from the thorax, abdomen and upper parts of the legs. With a knife heated in the flame, sear a broad line extending down the middle of the abdomen. Through this burned surface make an incision through the muscles of the abdomen. In a similar manner make a transverse incision across the middle of the abdomen through a burned surface. Cultures should be made from the peritoneal cavity, and smears upon cover-glasses prepared, which are afterwards to be stained. With a hot knife, scorch a small area on the surface of the liver; through this surface enter the liver with a sterilized platinum wire, and with the material withdrawn inoculate the tubes; also make cover-glass preparations. In the same manner inoculate tubes and make cover-glass preparations from the spleen, the kidneys, the pleural cavity, the pericardial cavity, the lungs, and the blood inside the heart. All incisions are to be made through the burned surfaces, and all material collected for inoculation is to be obtained through burned surfaces. Every time a knife or scissors has been used for cutting any structure it should be sterilized in the flame. In sterilizing the instruments avoid sputtering, especially when they become covered with oil from adipose tissue. Pieces of lung, liver, spleen, kidney and other organs, as may be indicated, should be placed in 95 per cent. alcohol for fixation and hardening. The animal and the board on which it was extended should be burned, and the cage or jar and the instruments used should be sterilized by steam. The hands of the operator should be washed thoroughly with soap and water and with a 1–1000 solution of bichloride of mercury.

CHAPTER VII.

COLLECTION OF MATERIAL.

SAMPLES of water or milk collected in sterilized tubes or bottles, when they are not examined immediately, or when they are to be transmitted any distance, should be kept on ice. Specimens of sputum may be collected in clean bottles tightly corked. They should be examined as soon as possible. Although decomposition appears not to interfere with the staining properties of the tubercle bacilli, the sputum should be fresh in order that the other bacteria contained in it may be studied. Therefore it should be free from contamination with putrefactive germs. Furthermore, valuable information can also be obtained by examination of the sputum in a fresh condition before staining (see also page 30).

Samples of urine keep better after the addition of a few crystals of thymol, which retards fermentative processes, so that the sedimentation of the bacteria and of other solid matter in conical vessels is facilitated, although that purpose can be accomplished at once by the centrifuge. Thymol will also be a useful addition, as far as a bacteriological examination is concerned, in case samples of urine are to be sent by mail, provided that no cultures are to be made.

Specimens of sputum, pus or blood may be collected conveniently in the form of thin smears upon cover-glasses. The smears are fixed by passing through the flame three times. Smears of blood are prepared as follows: Have two perfectly clean, square cover-glasses. The finger, or the lobe of the ear, having been carefully washed with water, alcohol, and ether, is punctured with a sterilized needle, and a small drop of blood issues which is wiped away. The second drop of blood should be taken; it should be about the size of a pin's head. No pressure on the finger should be used. This drop of blood is placed on one of the cover-glasses. The other cover-glass is laid upon the first, both being handled with forceps. The drop of blood becomes flattened out into a thin film. Immediately and before the blood has had time to coagulate the two are drawn away from each other in a horizontal plane, not forcibly pulled apart. The blood, therefore, will be spread in a thin film on the cover-glasses. It is best to place the cover-glasses so that one does not cover the other exactly, but so that the sides of the one lie at right angles with the sides of the other, although their centers coincide (Fig. 35). Films of blood which are to be examined for the parasite of malaria may be prepared in this manner. Samples of blood to be used for the Widal reaction for typhoid fever need to be pretty good-sized drops of blood, which may be collected on cover-glasses or pieces of paper and allowed to dry. To test blood by culture methods, rarely as much as 1 c.c. is drawn from a vein during life, using a sterilized hypodermic syringe and all antiseptic precautions.

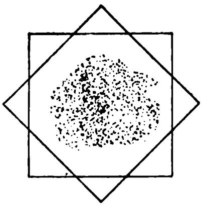


FIG. 35.—Manner of Placing Cover-glasses in Making Films of Blood. (After Cabot.)

At Autopsies on human subjects plate-cultures should be made, if possible, directly from the organs. In all cases organs should be entered by the platinum wire through a burned surface. The method of isolation by streaking the platinum wire containing the material under examination lightly, several times, over the surface of an agar plate, will be found convenient. At the same time smears should be made from the organs upon cover-glasses for microscopical study, and portions of the organs should be saved and hardened in alcohol.

A convenient device for the collection of infected material is a stiff wire wound with a pledget of absorbent cotton at one end, the whole sterilized in a tube, as recommended by Warren for collecting pus and other fluids for examination, and as introduced by W. H. Park for the collection of material from the throat in cases of suspected diphtheria.

The so-called Sternberg bulb is valuable for the collection of fluid materials for examination. A short piece of glass tubing is taken; at one end is blown a bulb; the other end is drawn out to a long, fine point. To introduce the substance into the bulb, the expanded end is heated in the flame; the point is broken and introduced below the surface of the fluid which is to be collected; as the bulb cools, the air in it contracts and draws the fluid into it. When it has taken up as much as it will, the point may again be closed in the flame.

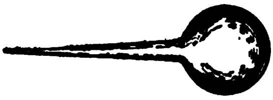


FIG. 36.—Sternberg Bulb.

Concerning the transmission of materials containing bacteria in the mails, the ruling of the post-office department of the United States, January, 1898, is as follows:

"That the order of the Postmaster General of June 1, 1893, forbidding the use of the mails for the transmission of specimen germs of cholera or other diseased tissues, is hereby modified to this extent:

"Specimens of diseased tissues may be admitted to the mails for transmission to United States, State or municipal laboratories, only when inclosed in mailing packages constructed in accordance with the specifications hereinafter enumerated. Upon the outside of every package shall be written or printed the words: 'Specimen for bacteriological examination.' No package containing diseased tissue shall be delivered to any representative of any of said laboratories until a permit shall have first been issued by the Postmaster General, certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens."

The specifications prescribing the manner of packing, which are minute and complicated, may be obtained from local postmasters. See Postal Guide, January, 1898, ruling No. 82, page 901.

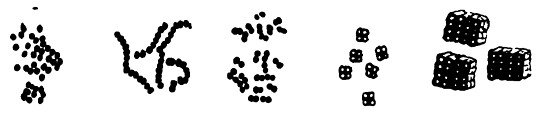
PART II.

CHAPTER I.

CLASSIFICATION; GENERAL MORPHOLOGY AND PHYSIOLOGY OF BACTERIA.

THE relationships existing between bacteria and other kinds of organisms are not perfectly clear. It is quite generally conceded, however, that bacteria are plants. They show affinities with both the lower algæ and the lower fungi, and they have even some points of resemblance with certain of the protozoa. On account of their extreme smallness it is impossible to analyze the structure of the individual bacteria and to contrast the structure of one with that of another. The classification cannot therefore be established on anatomical grounds, as is done with large animals or plants, but we are obliged to rely upon their behavior on culture-media, the appearances of the growths, and the production of certain substances with peculiar chemical reactions, when we wish to establish the points of difference between one species and another—all of which is extremely unsatisfactory and probably not perfectly reliable. It is likely that forms which are now considered as different species are not really such in all cases, and also that different species may now be included under one heading as a single species. There are even said to be a few species of bacteria that possess chlorophyll. The majority of bacteria have no green coloring matter. Notwithstanding the unsatisfactory condition of the classification of bacteria, it must not be supposed that the species of bacteria are not permanent. For instance, it would be incorrect to imagine that it is possible for the micrococci and spirilla to become converted into species of bacilli, or for the bacilli of one species to be transmuted into those of another. This does not contradict the statement that we may frequently, through erroneous and imperfect information, be in the habit of including unlike species under one name, or of classifying mere varieties of one species as entirely different species. At present the simple division of bacteria into three great generic groups is as good as any: *micrococci*, spherical forms; *bacilli*, rod-shaped forms, one diameter being in excess of the others; *spirilla*, twisted like a corkscrew, making long spirals or simply parts of spirals (comma-shaped forms).

The micrococci are subdivided into *staphylococci*, where



Staphylococci. Streptococci. Diplococci. Tetrads. Sarcinae.

FIG. 37.

the spheres grow in clusters like a bunch of grapes; *streptococci*, where they are arranged in long rows or chains, like a string of beads; *diplococci*, or pairs of micrococci; *tetrads*, where the individual spheres are grouped in fours; *sarcinæ*, where they are grouped in eights, making the outline of a cube, resembling a bale or package tied with rope.

The bacilli are not usually subdivided in this manner, although their forms vary considerably. The ends are sometimes square, sometimes round. Sometimes they are very short, and sometimes they grow in longer, thread-like forms, in which, however, the transverse markings which indicate the outlines of the individual bacilli can generally be seen. Short oval bacilli may look exceedingly like micrococci. Under exceptional circumstances, branching forms of the bacilli of diphtheria, tuberculosis and glanders have been encountered.

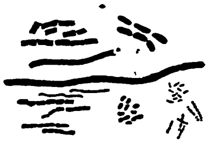


FIG. 38.—Bacilli of various forms.

The word "*bacterium*" was formerly used to designate short bacilli which generally formed no spores, while the word bacilli was restricted to the longer forms in which spore formation occurred. This use is no longer common, although not rarely the name bacterium is still given to a species—for instance, bacterium coli commune.

Spirilla present a very great variety of form. The short "*comma-shaped bacilli*" are only parts, at most, of spirals, although the microbes of cholera do sometimes form long spirals. On the other hand, there are among spirilla large and long sinuous figures which present most remarkable pictures under the microscope; for example, the spirillum of relapsing fever. Formerly spirilla without very marked windings were called "*vibrios;*" and long, wavy forms with corkscrew-like windings "*spirochœtae;*" and the rigidly spiral forms were denominated "*spirilla.*" These definitions have for the most part lost their significance, although the names still linger in nomenclature.

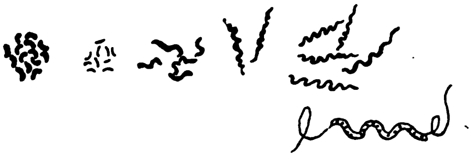


FIG. 39.—Spirilla of various forms.

Beside the classification already mentioned, bacteria are sometimes grouped according to certain other qualities. In general botany, saprophytes are plants that grow on decaying vegetable matter. In a bacteriological sense, *saprophytes* are bacteria which grow in external nature on dead organic matter, and *parasites* are bacteria which exist upon the living tissues or fluids of any organism. Nearly synonymous with the above words are those which do not and those which do produce disease, or *non-pathogenic* and *pathogenic*. The adjectives *facultative*, or optional, and *obligate*, or strict, are used to qualify the above terms and many others.

Size.—Bacteria vary greatly in size. The micrococci are usually 1 μ or less in diameter. The short diameters of bacilli and spirilla also are less than 1 μ as a rule, while the length may be several μ. The anthrax bacillus (1.5 μ × 3 to 10 μ) and the spirillum of relapsing fever are the largest bacteria known to be pathogenic to man. To say that a micrococcus is 1 μ in diameter means that 25,000 end to end would make a line 1 inch long. It has been estimated that 1 milligram of a pure culture of the staphylococcus pyogenes aureus contains 8,000,000,000 micrococci.

In stained preparations the bodies of bacteria frequently seem to be homogeneous. On the other hand, they may exhibit certain spots which stain more intensely than others, the stained spots alternating with clear areas representing vacuoles.

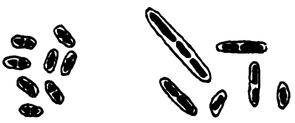


FIG. 40.—Bacteria with capsules.

In old cultures bacteria are likely to show deformed and twisted outlines called *involution forms* (Fig. 63). It is not uncommon for bacteria to be enclosed in a kind of envelope of some clear substance, which stains with difficulty or not at all, called a *capsule* (Fig. 40). The paired micrococci of pneumonia are enclosed in such capsules. The capsule is more likely to be demonstrated when the bacteria are obtained from the fluids derived from the animal's body than when they have been grown artificially in culture-media. A *zoöglœa* is a large mass of bacteria held together by a mucilaginous substance.

The composition of bacteria varies considerably with different species. The basis appears to be an albuminous substance called *mycoprotein.*

The multiplication of bacteria takes place in almost all cases by transverse fission. The formation of tetrads or sarcinæ from micrococci depends upon fission in two or three planes. Repeated fissions of micrococci in one plane result in the formation of streptococci. Micrococci that have recently divided are likely to be somewhat flattened. Multiplication under favorable circumstances may take place at a phenomenally rapid rate. Bacilli have been observed to divide in twenty minutes. If division takes place once in an hour, the progeny of one organism at the end of twenty-four hours will be 16,772,216, *i.e.*, $(2 \times 1)^{24}$. The ordinary form of reproduction by fission is called *vegetative*, and bacteria that are multiplying in this manner are often spoken of as being in the vegetative condition.

Spores.—Under certain circumstances the reproduction of bacteria takes place by means of bodies called *spores.* They appear in a typical form in the large bacilli, where, near the centers of the bacilli, highly refracting, shining spots may be seen which are found to stain less rapidly with the aniline dyes than the rest of the bacilli (Fig. 68). They are not to be confused with the unstained spots described as vacuoles. On account of their being formed from a part of the interior of the bacterium, such spores are called *endogenous.* These spores are found mostly in the bacilli, rarely in spirilla, and in micrococci. They are what is meant when the word spore is used alone without qualification. The existence of another kind of spore, described as forming from micrococci chiefly (called *arthrospore*), is doubtful. At all events, its significance is not at present understood. Spores develop mostly though not always under adverse conditions of various kinds, as of temperature and of nutrition. They are more resistant to unfavorable influences of all sorts than are the fully-developed bacteria. Spores resist drying, light, heat and chemical agents to a remarkable degree, at times.

Anthrax spores are said to have been found which could withstand steam for twelve minutes, 1–1000 mercuric chloride for nearly three days, or 5 per cent. carbolic acid for more than forty days. The greatest resistance is displayed by the spores of some of the saprophytic bacteria, particularly those of hay and potato, which are sometimes not destroyed by several hours of steaming; and bacteria are said to have been obtained from the soil which resisted 100° C. for sixteen hours. When cultivated at a temperature as high as 42° C. the anthrax bacillus becomes incapable of forming spores. Spores themselves do not multiply, nor do they manifest any activity. Spores may be located at the center of the bacillus, or nearly at one end when the end of the bacillus is likely to enlarge, making a form having the shape of a drumstick, as takes place with tetanus bacilli. When a bacillus assumes a spindle shape on account of having the middle part bulged through the formation of a spore it is called a *clostridium*. Under favorable conditions the spores germinate, as it is called, and develop to the adult form of the organism. This may be witnessed in hanging-drop preparations.

Motility.—Motility is rarely exhibited by micrococci; some bacilli possess it and some do not; while nearly all of the spirilla are motile. The phenomenon is observed in the hanging-drop. The degree of motility is variable, being sometimes slight and sometimes very active. When seen under a high power the little particles taken from a culture of a motile organism may look like a writhing mass of maggots or like tadpoles in a pool. The motility is most active in young cultures. The movement results from the vibration of little processes, or *flagella* (Fig. 72). Of these there may be one or several. They may be placed singly or in groups, at the end, or scattered around the sides. They are extremely difficult to demonstrate except by special staining methods, which, furthermore, are decidedly capricious. After the flagella have been stained, the bacteria appear somewhat larger than when stained by the ordinary methods. The flagella upon the bacilli of typhoid fever are numerous and form a very striking picture.

Chemotaxis.—Motile bacteria possess the property of being attracted by certain substances (positive chemotaxis) and of being repelled by others (negative chemotaxis). Similar properties are widely distributed among living cells, both animal and vegetable.

CONDITIONS FAVORABLE FOR THE GROWTH OF BACTERIA.

Warmth.—Among the different kinds of bacteria forms are said to exist which are known to multiply at points all the way from 0° C. to 70° C. Bacteria which flourish at a very high temperature (maximum 70° C.) are called *thermophilic.* The pathogenic bacteria usually flourish better at a point somewhere near the temperature of the human body. This is not necessarily the case with the non-pathogenic species. Ordinary water bacteria thrive better at ordinary temperatures.

Sternberg's method for determining the *thermal death-point* of a species of bacteria is to draw portions of a pure culture of the organism into capillary tubes with expanded ends, and the tubes are sealed in the flame. The tubes are supported upon a glass plate placed in a water-bath, whose temperature is indicated by a thermometer, while a uniform temperature is secured by stirring. The time of exposure is, as a rule, ten minutes.

Moisture is indispensable to the growth of bacteria, and drying causes the death of certain kinds, as, for instance, the spirillum of cholera.

Food.—On account of the absence of chlorophyll, bacteria require, as part of their food, organic compounds containing carbon, such as sugar. They are unable, with possibly a very few exceptions, like the nitrifying bacteria, to derive their carbon from the carbon dioxide of the atmosphere, or from inorganic carbon compounds. Although some species are able to obtain nitrogen from inorganic salts, most bacteria flourish best if organic substances containing nitrogen, like peptone and albumen, are furnished them as part of their food. The complicated, unstable, organic molecules with high potential energy are converted by them into the simple and more stable compounds like carbon dioxide, ammonia and water, with the liberation of energy. These facts become manifest in connection with their important work in decomposition, putrefaction and fermentation. A culture-medium having a slightly alkaline or neutral reaction is favorable to most bacteria.

The prolonged artificial cultivation of bacteria may or may not modify their properties. The pathogenic bacteria are likely to undergo considerable modification both in the quality and luxuriance of their growth and the intensity of their pathogenic characters.

The growth of bacteria may eventually be hindered by the accumulation of the products of their own metabolism. Many bacteria refuse to grow on culture-media at all. Some species are extremely fastidious, and can only be propagated on particular sorts of nutrient substances.

Relation to Oxygen —Oxygen is indispensable to the existence of some bacteria, *aërobes.* Its absence is equally indispensable to certain others, *anaërobes.* Others still are able to flourish either in the presence or absence of oxygen, facultative aërobes or anaërobes. The first-named varieties are sometimes called strict, or obligate aërobes or anaërobes.

Effects of Sunlight.—Direct sunlight kills the vegetative forms of bacteria more or less rapidly, and constitutes one of the most efficient among the natural methods of disinfection. Diffuse light acts much more slowly. Spores are affected less or not at all.

The influence of *electricity* upon bacteria has not yet been fully studied. Apparently the destruction of bacteria reported as having been effected by electricity was the result of electrolysis.

CHAPTER II.

PRODUCTS OF THE GROWTH OF BACTERIA.

Phosphorescence.—Bacteria, whose cultures exhibit phosphorescence, have been found in the ocean and in fish.

Chromogenic Bacteria.—Many bacterial growths display brilliant coloring. The different species of sarcinæ are remarkable for forming highly-colored growths; some of them are rose-red, some orange-yellow, some lemon-yellow, and so on. The micrococcus prodigiosus presents a brilliant red growth whose rapid development is said to have formed the basis for the so-called "Miracle of the Bleeding Host" (see page 13). The bacillus pyocyaneus in cultures gives a brillant green fluorescence and is responsible for the color of blue or green pus.

Bacilli which exhibit a green fluorescence in cultures are common in water. In cultures on potato or agar the colors of the chromogenic forms are usually well shown.

Ferments or Enzymes.—Many bacteria form ferments which have the power of dissolving proteid substances in a manner similar to trypsin. The liquefaction of gelatin is a familiar example of this process. The property of liquefying gelatin, or otherwise, is used in classifying bacteria and in determining the nature of unknown species.

Some bacteria, as the bacillus coli communis, form ferments which act like rennet in coagulating milk. Other bacteria act like diastase, forming glucose from starch. Others have the power of changing cane-sugar into glucose.

Bacteria which are able to decompose cellulose are found in the stomachs of ruminant animals. Although it is doubtful whether the products of cellulose decomposition have any nutritive value, the process is useful in effecting a subdivision of the coarse food, consisting of grass, hay, and the like.

Some bacteria have the power of decomposing neutral fats into fatty acids and glycerin, after the manner of the fat-splitting ferment of the pancreatic juice.

The end-products which result from the growth of bacteria upon albuminous nutrient media are very numerous. They are complicated and not well understood. Among these end-products may be mentioned peptone, indol, skatol, phenol, leucin and tyrosin. Nearly related are the ptomaines and toxins, which play an important part in the production of disease by pathogenic bacteria. In the decomposition of urine by bacteria the urea is converted into ammonium carbonate.

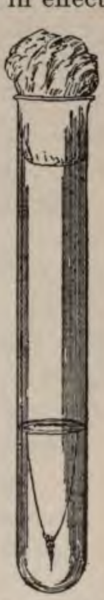


FIG. 41.—Culture of Staphylococcus pyogenes aureus showing liquefaction of gelatin.

The *formation of indol* in cultures is an important peculiarity of certain bacteria. The test is as follows: The bacteria are cultivated in Dunham's peptone solution; after twenty-four to forty-eight hours the test may be made. Add ten drops of concentrated sulphuric acid; the development of a rose-color indicates the presence of both indol and nitrites. If no rose-color forms, to another tube add, first 1 c.c. of a 0.01 per cent. solution of sodium nitrite, and then the sulphuric acid. The development of a rose-color indicates the formation of indol but not of nitrites. If there is no rose-color, no indol has been formed. The color appears usually in a few minutes, but it may only develop after a somewhat longer time. Control tests must be made upon tubes of the same peptone solution but which have not been inoculated. The reaction may be hastened by warming slightly. The value of this reaction will be understood when, to give one illustration, it is remembered that the bacillus coli communis produces indol and the bacillus of typhoid fever does not. The reaction depends upon the liberation of nitrous acid, which, with indol, forms a red color.

The change of organic substances into more stable ones does not cease with the compounds mentioned above. Certain bacteria of the soil which will be discussed further on are able to complete the conversion of ammonia into nitrous acid (leading to the formation of nitrites); and others still that of nitrites into nitric acid, which at once forms nitrates.

Formation of Acids.—In the course of their growth many bacteria produce acids, especially from substances containing sugar. The power of developing lactic acid is possessed by a large number of species. Acetic acid is another common by-product. Besides these, butyric acid, formic acid, propionic acid and many more are formed by different bacteria.

Development of Gas.—The evolution of gas from bacterial growths is of frequent occurrence. Carbon dioxide, hydrogen sulphide and nitrogen are among the better-known gases that may be formed. The odors that arise from cultures and that are so characteristic of putrefactive processes depend upon the development of gases, or a mixture of gases, of considerable complexity. The bacillus aërogenes capsulatus leads sometimes to the formation of gas in the organs of the human cadaver within a short time after death. T. Smith considers the formation of gases in media containing sugar of importance in discriminating between different species. Bouillon containing 1 per cent. of dextrose, lactose or saccharose is the culture-medium advised.

The test is best conducted in a U-shaped tube, closed at one end, and at the other end provided with a bulb (Fig. 42). The tube is stoppered with cotton, sterilized by dry heat, afterward filled with the bouillon, and sterilized by steam in the usual manner. After the last sterilization it should be tilted until the closed end is completely filled with the medium. After it has been inoculated with the species under consideration, any development of gas will be indicated by the collection of the gas at the closed end. The amount of gas formed may be estimated and its quality tested. The addition of a 2 per cent. solution of sodium hydrate, followed by shaking, causes the absorption of the carbon dioxide and diminution in the quantity of gas. The portions which remain may be mixed with air and ignited, when the presence of hydrogen and some of its compounds will be indicated by an explosion.

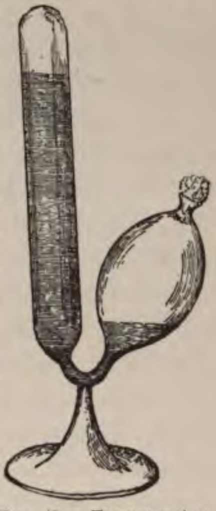


FIG. 42. — Fermentation-tube.

The development of gas may readily be tested by inserting the bacteria by a deep puncture into agar containing 1 per cent. of dextrose. The development of gas causes bubbles to form in the agar, often to the extent of splitting it, and sometimes forcing out the cotton plug.

The activities of bacteria which have just been enumerated are fundamental to the phenomena which go by the names of *fermentation* and *putrefaction*. These words have been defined differently at different times and by different writers, but in general both are used as names for the breaking up of complex organic compounds by microörganisms with the formation of simpler compounds. Fermentation refers especially to the formation of useful products like alcohol. The term putrefaction is employed chiefly for the breaking up of nitrogenous compounds with the development of foul-smelling gases. Fermentation is also the term applied to the decomposition of complex substances through the influence of unorganized ferments or enzymes.

The work of bacteria in fermentation and putrefaction is indispensable to the existence of the organic world as we find it. While green plants convert the stable compounds of nitrogen, the carbon dioxide of the atmosphere, and water into the complex and unstable albumens and carbohydrates which serve as food for animals, animals, on the other hand, have to convert these unstable and complex compounds back into simpler forms. The work of changing them back into the simple and stable condition, in which they serve as the food for plants, is performed by animal life in part only, and its completion is left to the activities of bacteria. It is the work of bacteria in this direction which we call fermentation and putrefaction. Without that work, as we understand it, the existence of life upon the earth would soon come to an end, and the dead and undecomposed bodies of living things and their products of all kinds would lie about unchanged, as they had fallen.

Bacterium termo is the name formerly given to a supposed species of bacteria which was credited with being the producer of putrefaction. The individuals were represented as being short rods, mostly going in pairs, and actively motile. The term has been abandoned since it appears to have included a number of different species.

CHAPTER III.

SYSTEMATIC STUDY OF A SPECIES OF BACTERIA.

In order to conduct the study of any species of bacteria it is necessary to have the organism isolated in a pure culture by the plate-method, or by some other method already described. Having thus obtained the organism in pure culture, it is to be examined with reference to its behavior in certain particulars. It is well for the beginner to study a few known species of saprophytes obtained from some reliable laboratory in pure culture. The points which are to be considered can be illustrated best by presenting them in tabular form, filling out the items of the table for a given species of bacteria.

1. Name.
2. Habitat or source.
3. Morphology, Grouping.
4. Size.
5. Spore formation.
6. Motility, Flagella.
7. Staining properties. Behavior by Gram's Method.
   Growth on culture-media.
8. Gelatin. Colonies in gelatin plates.
9. Agar.
10. Bouillon.
11. Milk.
12. Potato.
13. Blood-serum.
14. Relation of growth to temperature.
15. Relation to oxygen.
16. Production of gas.
17. Production of acid.

18. Production of indol.
19. Pathogenesis.

In commencing the study of bacteriology the pupil should perform the different manipulations described in Part I., including in particular the manufacture of the most important culture-media. Having culture-media prepared, it is customary to study a number of species of non-pathogenic bacteria. It is well to choose species which have properties decidedly different from one another. The micrococci, bacilli and spirilla should be represented; forms that are motile and that are not; species that form spores and others that do not form spores; some that liquefy gelatin and some that do not. There should be chromogenic forms, and species that ferment dextrose, and that produce indol,—such species as some of the sarcinæ, the bacillus coli communis, the hay bacillus, the potato bacillus, bacillus prodigiosus, a bacillus fluorescens and spirillum rubrum. It is well, when possible, to obtain material directly from nature rather than from laboratory, stock, pure cultures. This may readily be done in the case of the hay bacillus and the potato bacillus. Fecal matter may be spread on gelatin plates and the bacillus coli communis obtained in pure culture. Fluorescing bacilli are very common in water. Some organisms like spirillum rubrum can only be had from laboratory cultures. The pyogenic bacteria, which can easily be isolated from pus, may be studied in this connection with great advantage. The staphylococcus pyogenes aureus and the streptococcus pyogenes should on no account be omitted. The diplococcus of pneumonia can most readily be obtained from a mouse or a rabbit which has died with pneumococcus infection. It can be introduced most easily by subcutaneous insertion, using some of the rusty sputum of a case of lobar pneumonia. The cultivation of the pneumococcus will be found to present difficulties in classes containing large numbers of students.

Representative forms of moulds and yeasts should be studied at the same time. Moulds are easily obtained by exposing some nutrient substance to the air, covering it, and allowing cultures to develop. Yeasts will probably grow at the same time. Ordinary brewer's yeast may be isolated in pure culture from gelatin plates. Bacteriological examinations also should be made of air, soil, water and milk. With such simple means, all the important properties of bacteria may be demonstrated.

Experiments in sterilization and disinfection as described in Chapter IX., Part II., may be performed with the bacteria mentioned, which present every variety of resisting power up to the almost incredible toughness of the spores of the hay and potato bacilli. After some proficiency has been acquired, various pathogenic bacteria may be studied as the circumstances of the case require. Some judgment has to be used in allowing students to work with pathogenic bacteria. Anthrax, glanders, tetanus, cholera and diphtheria all have occurred in laboratory workers through accidental infection, sometimes with fatal results. The most important precaution, perhaps, is observance of the rule that while working in the laboratory, nothing should be put in the mouth. Cultures of pathogenic bacteria should be thoroughly sterilized before the tubes are cleaned. The writer is in the habit of having tubes containing pathogenic bacteria placed in the steam sterilizer for an hour on each of three days, and of having the plugs removed and burned and the tubes filled with 5 per cent. carbolic acid between the second and third sterilizations. In taking these measures, the same kind of reasoning applies as that which induces engineers to give bridges from four to six times the strength they need to bear the greatest strain likely to be put upon them, or to make the boiler of a steam engine strong enough to bear six times the greatest pressure which it is expected that the steam contained in it will exert.

CHAPTER IV.

DISTRIBUTION OF BACTERIA.

The Bacteria of the Soil.—Bacteria are present in the soil in enormous numbers—100,000 or more in 1 c.c. of virgin soil, according to Flügge. The depths to which they penetrate will depend upon the character of the soil and the character of the life upon it, and whether or not it has been artificially disturbed, as by cultivation. In general, at a depth of 1.25 meters (about four feet) the number will have become very small, and a little deeper the soil will be entirely sterile.

The bacilli of tetanus and malignant edema are present in the soil of many localities. According to Woodhead, certain savage tribes of Africa and the East Indies use as an arrow-poison soil that is capable of producing tetanus. The bacillus of anthrax may be found in soil which has been infected with this organism. It is possible that the organism of malaria, which is not bacterial, but a protozoön, may exist in the soil.

Most of the bacteria of the soil are harmless and useful saprophytes. The nitrifying bacteria of Winogradsky belong to this class. An organism called nitrosomonas has been discovered which possesses the power of converting ammonia into nitrous acid which forms nitrites, while another, the nitromonas, completes the change of nitrites into nitrates. The nitrifying bacteria will not grow upon the ordinary culture-media, and their cultivation presents great difficulties. Bacteria have been credited with the assimilation of free, atmospheric nitrogen, resulting in the addition of a valuable proportion of nitrogen compounds to the soil. Inasmuch as a large part of the excrementitious products of animals containing nitrogen are not retained in the soil, where they may be employed as food by plants, but are washed directly or indirectly into the sea by means of sewage and the rivers, it will be seen that the supply of nitrogen compounds might be in a way to suffer gradual exhaustion. Furthermore, it has already been noticed (Chapter II., Part II.) that one of the products of decomposition by bacteria is nitrogen, which is not available to animals and most plants as food. These facts have met with practical recognition by agriculturists in the adoption of various methods of fertilizing the soil. It appears that the roots of peas, beans and other leguminous plants frequently present minute tubercles. These tubercles are pathological growths, caused by the development of microörganisms related to the bacteria. The organism, called by Prazmowski bacillus radicicola, appears to have the power of assimilating atmospheric nitrogen and of converting it into nitrogen compounds. Experiments show that these observations may be destined to be of great value to the farmer.

The bacteria of the soil may easily be studied in plate-cultures made from small portions of soil collected with the necessary precautions to avoid contamination, or plate-cultures may be made from sterilized water with which a portion of the soil has been properly mixed. Anaërobic bacteria must be cultivated by the special methods adapted to them.

Bacteria of the Air.—The bacteria of the air will be found for the most part clinging to solid particles in suspension in the shape of dust. As has already been stated, bacteria will not rise from moist surfaces unless forcibly removed, as by agitation or currents of air. Conditions of dryness and wind tend to increase the number of microörganisms in the air. They are fewer after a fall of rain or snow, and the number is smaller in winter than in summer. The air of cities contains more germs than that of the country. The atmosphere over the sea and at the tops of high mountains is nearly or wholly free from germs. The bacteria which do occur in the air will seldom be pathogenic. Their character will depend upon the character of the dust. It is obvious that dust which consists in part of the dried, pulverized expectoration of cases of pulmonary tuberculosis may contain tubercle bacilli. Anthrax of the lungs sometimes arises in men who handle the wool of sheep that were infected with anthrax (Wool-sorter's disease), and is due to the inhalation of anthrax spores attached to the wool. It is likely that the atmosphere in the immediate vicinity of cases of the exanthematous fevers may contain the organisms, whatever they may be, that cause these diseases. Malaria is probably conveyed through the atmosphere in some unknown manner.

In a rough way one may obtain some knowledge of the character of the organisms in the air of a given locality by removing the cover of a Petri dish containing sterilized gelatin or agar for a few minutes, replacing it, and allowing the organisms to develop. In most cases a large proportion of the growths that appear will be moulds. Yeasts are also common, and among the bacteria the micrococci are abundant. Chromogenic varieties are likely to be present.

A few studies of this character will show that the number of organisms that are present depends chiefly upon whether the air is quiet or has recently been disturbed by draughts, gusts of wind, or sweeping. These facts are of fundamental importance in laboratory work, where plate-cultures are being studied, if we wish to avoid contamination of the plates. Among various devices that have been proposed for the accurate study of the organisms of the air, the Sedgwick-Tucker aërobioscope is the simplest and most accurate. It consists of a glass tube, one end of which is drawn out so as to be smaller than the other. The small end contains a quantity of fine granulated sugar; both ends are plugged with cotton, and the instrument is sterilized. A definite quantity of air is to be aspirated through the large end, after removing the cotton, which may be done by means of a suction-pump applied to the other end, or by siphoning water out of a bottle the upper part of which is connected with the end of the aërobioscope by means of a rubber tube. The sugar acts as a filter and sifts out of the air the microörganisms which are contained in it. Liquefied gelatin or agar may be introduced into the large end of the instrument by means of a bent funnel; and, after replacing the cotton, it may mix with the sugar which dissolves. The culture-medium may be spread around the inside of the larger portion of the tube after the manner of an Esmarch roll-tube. The bacteria which were filtered out by the sugar will develop as so many colonies upon the solidified medium.

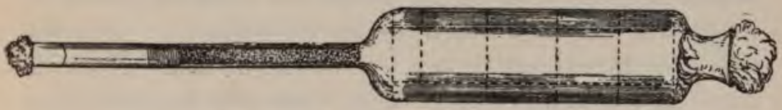


FIG. 43.—Sedgwick-Tucker Aërobioscope.

Bacteria of Water and of Ice.—The water of rivers, lakes and the ocean always contains bacteria. The number of organisms varies greatly in different places and under different conditions. The number of different species found in water is also very large. Ground-water* contains few or no bacteria under normal conditions, and is therefore suitable for a source of water-supply, when a sufficient amount is available. The possibility of contamination of the ground-water from unusual or abnormal conditions should always be eliminated before it is taken for drinking-water.

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* Ground-water is the water which—originally derived from rain or snow—sinks through superficial porous strata, like gravel, and collects on some underlying, impervious bed of clay or rock.

The ordinary bacteria of water are harmless, as far as is known. The diseases most commonly disseminated by water are typhoid fever and Asiatic cholera. The spirillum of cholera will usually die in natural water (not sterilized water) inside of a week; the bacillus of typhoid fever will usually die in two weeks. Under exceptional circumstances these organisms may perhaps maintain their vitality for a longer period. They appear, however, to be less hardy than the ordinary water bacteria. As we now understand these diseases, the organisms causing them will be present only in a water-supply which has been contaminated by the excreta from a case of the disease. Notwithstanding the rapid death of these organisms in water, they may exist long enough to infect individuals habitually drinking the water. Many epidemics of cholera and typhoid fever have been traced to the discharges from cases of these diseases.

By *self-purification* of water is meant the removal through natural processes of contaminating organisms such as might occur from the discharge of sewage into it. It depends upon the deposit of the contaminating material, in the form of mud, upon the growth of the ordinary water-plants and bacteria (including the work of the nitrifying organisms), and upon the destructive influence of direct sunlight, after the dilution of the matter added with a large volume of water. It is not usually to be relied upon as a means of freeing the water-supply from pathogenic bacteria.

Filtration.—Filtration on a large scale is more commonly in use in the continental cities of Europe than elsewhere.

The filter consists of successive layers of stones, coarse and fine gravel. The uppermost layers are of fine sand. The whole filter is from 1 to 2 meters thick. The sand should be 60 cm. in thickness. It may be removed from time to time, not becoming less than 30 cm. in thickness. The first water coming from the filter is discarded. The actual filtration is done by the slimy sediment which collects on the surface of the layer of fine sand. The filter-beds may be several acres in extent, and are protected by arches of brick or stone. They require renewal occasionally. This kind of filtration has come largely into use since the cholera epidemic of 1892–93, and it appears to be very effective. For other methods for the purification of water-supplies consult the works on hygiene.

The filtration of water on a small scale, as is ordinarily done for domestic purposes, is usually entirely useless. The so-called Pasteur filter of unglazed porcelain is effective if it is properly constructed and if the filter-tubes are sterilized by heat frequently (every few days)—conditions which are seldom complied with.

Collection of Samples.—Samples from the water-supply of a city may be drawn from the faucet, but the water should first be allowed to run for half an hour or longer. From other sources the supply should be collected in sterilized tubes or bottles, taking care to avoid contamination. Sternberg bulbs (see page 85) will be found useful for small samples. These samples should be examined as quickly as possible, for the water bacteria increase rapidly in number after the samples have been collected. When transportation to some distance is unavoidable the samples should be packed in ice.

The **number of bacteria** may be determined by making plates of a definite quantity of the water with gelatin or agar. The amount examined ordinarily is 1 c.c. When the number of bacteria is very large, a smaller quantity must be taken, and it may be necessary to dilute the sample ten times or more with sterilized water. The amount should be measured with a sterilized, graduated pipette. The water is to be mixed with liquefied gelatin or agar in a tube which has been allowed to cool after melting. After thorough mixing, remove the plug, burn the edge of the tube in the flame, hold in a nearly horizontal position until cool, and pour into a sterilized Petri dish. The number of colonies may be counted on the third or fourth day; the later the better, as some forms develop slowly and may not present visible colonies for several days; but the plates are often spoiled after three or four days by the profuse surface growths of certain forms or by the rapid liquefaction of gelatin, if that be used, by other forms. The number of colonies that develop is supposed to represent the number of individual bacteria contained in the quantity measured. That will probably not always be the case, however, as colonies may develop from a clump of bacteria which have not been separated from one another by the mixing process. Abbott has shown that the number of colonies is usually larger on gelatin plates than upon agar plates, and at the room temperature than in the incubator. This observation illustrates the fact that there are doubtless many kinds of bacteria that do not find favorable conditions for development on ordinary culture-media. The reaction of the medium

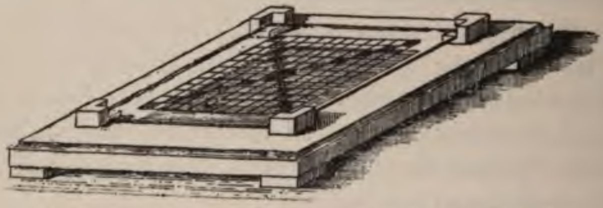

FIG. 44.—Wolffhügel Plate for Counting Colonies of Bacteria.

has an important influence upon the development of these water bacteria in plate-cultures.

When the number of colonies is small, there is no difficulty in counting them as they appear in the ordinary Petri dish. When the number is large, some kind of mechanical device may be used to assist counting. The Wolffhügel plate is a large square of glass resting in a wooden frame painted black (Fig. 44). The glass plate is ruled in squares. It was designed particularly with reference to the form of plate-

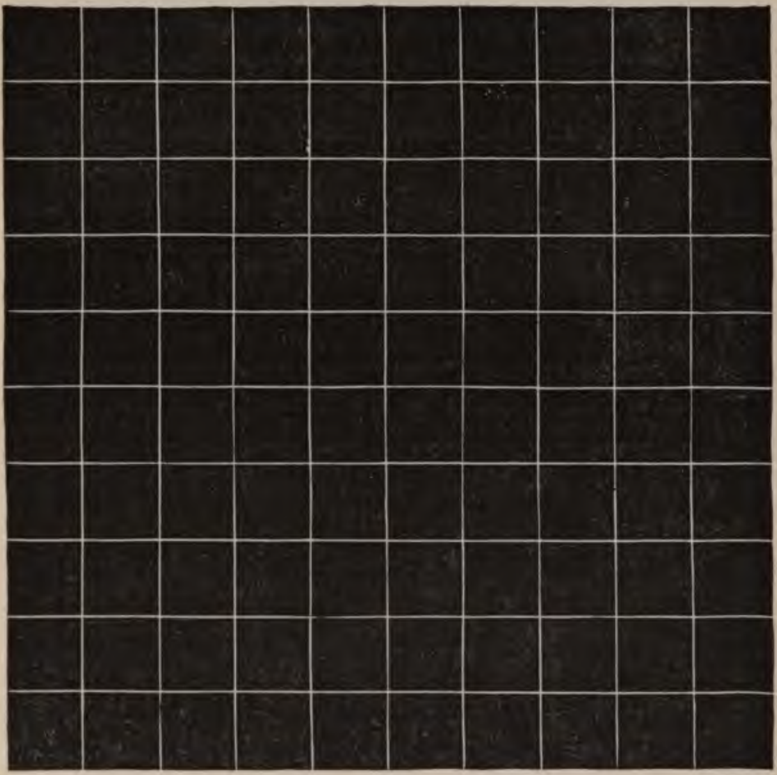

FIG. 45.—Surface Divided in Square Centimeters for Counting Colonies.

cultures first made by Koch. The Petri dish, however, may be placed upon the glass plate and the cross lines be used to assist in counting. Lafar and Parks recommend a surface painted black, ruled with white lines which represent the radii of a circle, which may be still further subdivided by other lines. Many find counting easier when a black surface divided into squares is employed (Fig. 45). An ordinary card with a smooth black surface divided into squares by white lines may be placed under a Petri dish and will be found to serve very well. For the mere examination of the colonies no better surface can be devised than the ferrotype plate used by photographers. The examination of the colonies will be easier if a small hand-lens be used. Care must be taken not to mistake air-bubbles or particles of dirt for colonies of bacteria.

In any case, if possible, all the colonies in the plate should be counted. The number contained within several squares may be counted and the average taken; knowing the size of the squares and the area of the plate, the number contained in the whole plate may be calculated. Such estimations, however, are likely to give results very wide of the truth.

The plating may be done by rolling the medium after the manner of Esmarch. When the number of colonies is not large this may serve very well. Counting may be assisted by drawing lines with ink on the outer surface of the test-tube.

It has been said that a water-supply containing no more than 500 bacteria per cubic centimeter is to be regarded as safe, one having between 500 and 1000 is to be looked upon with suspicion, and that where there are more than 1000 to the cubic centimeter the water is unfit for drinking purposes. It is obvious, however, that the character of the bacteria is of prime importance; that pathogenic organisms may occasionally be present, even when the number of bacteria to the cubic centimeter is small. But knowing the number usually found in a good water-supply, any sudden variation above that number is to be looked upon with suspicion. An increase is to be expected when the water has been subjected to unusual agitation from winds or currents.

The detection of pathogenic bacteria in water involves great difficulties, and our knowledge in this direction is very meagre. Koch and several others have reported finding the spirillum of Asiatic cholera in water. The examination of water-supplies for this organism has disclosed the fact that bacteria resembling the organism of cholera in many respects are not uncommon in water. This adds to the difficulty of detecting the cholera germ in water.

The bacillus of typhoid fever has many times been described as occurring in water-supplies suspected of being contaminated with the excreta of cases of the disease. The interpretation of these observations is at present doubtful. It is now known that several forms of bacteria exist which closely resemble the bacillus of typhoid fever, and which would make its recognition in an unknown specimen very difficult.

It will at once be appreciated that the number of cholera and typhoid organisms necessary to contaminate a considerable body of water, and sufficient to cause an outbreak of the disease among some of the people drinking the water, may still be so small that many different cubic centimeters of the water might be studied before a single one of the specific organisms would be encountered. Anyone who has examined plates made from samples of water will recognize the difficulty of detecting one or a few colonies of the bacteria of cholera or typhoid fever among a hundred or more colonies of ordinary water-bacteria. The existence of contamination with animal excreta might, however, be indicated by finding the bacillus coli communis, whose detection offers a greater prospect of success.

Certain devices have been adopted to hasten the development of the desired bacteria and to retard that of the ordinary water-bacteria. Among these may be mentioned the influence of the heat of the incubator, which will hasten the growth of organisms derived from the human body, and which retards the growth of water-bacteria. Another is the addition of a solution of peptone to a large quantity of the water to be examined with a view to assisting the development of the desired bacteria by furnishing them suitable food for growth. T. Smith recommends the addition of the water to tubes of bouillon containing dextrose and which are kept in the incubator. Suspected bacteria may be tested by inoculation into animals; the possession of pathogenic properties creates a probability in favor of their having come from some contamination with animal excreta.

Ice.—The bacteriological examination of ice differs in no respect from that of water. Although development may be arrested, the vitality of bacteria is not usually impaired by freezing. Prudden found the bacillus of typhoid fever alive in ice after more than one hundred days. The source from which ice is taken should be as carefully scrutinized as that of the water-supply, especially in view of the universal habit of cooling water in the summer time by adding ice directly to the water. It is better to cool water and articles of food by surrounding the vessels containing them with ice.

Bacteria of Milk and Other Foods.—Of the different food substances, milk is probably the most important from a bacteriological point of view. In the first place, most other foods are cooked before eating. Furthermore, cow's milk constitutes a large part of the food of many young infants who are highly susceptible to certain bacteria, or to substances in the milk itself, after it has undergone certain alterations due to bacteria. The milk of the healthy cow while still contained in the mammary gland is sterile; however, after milking the cow a little milk generally remains in the lower part of the teat in which numerous bacteria will have developed before the next milking-time. The first milk obtained at a milking should therefore be discarded, as it may contaminate the whole quantity.

Contamination with bacteria may occur from the outer surface of the udder of the cow, the hands of the milker or dirty pails, or through agitation of the air of the stable, and in other ways readily conceived of. Excluding the tubercle bacillus, the organisms which contaminate milk will be pathogenic only in exceptional cases. Occasionally typhoid fever, cholera, and possibly scarlet fever, diphtheria and other diseases are disseminated by means of contaminated milk. But the mixture with the milk of non-pathogenic organisms from the air, and their growth, may induce changes in it which render it unfit for consumption, and even poisonous. These alterations may be evident to the senses, as the ordinary lactic acid fermentation (souring of milk), or they may not. The character of the alterations doubtless varies much with the temperature and with the character of the contaminating bacteria. Summer temperatures of course favor decomposition and fermentation. Specialists in children's diseases attribute to alterations in milk with the formation of poisonous substances a preëminent influence in the production of the intestinal disorders of infancy so common in the summer.

Poisoning with milk, ice-cream or cheese is not rare, as is well known. There are many records of whole companies of individuals having been taken violently ill after having eaten one of these foods from the same source of supply. The symptoms in such cases resemble those produced by irritant mineral poisons such as arsenic: nausea and vomiting, vertigo, dryness of the mouth, sense of burning and constriction in the throat, difficulty in swallowing, cramps and griping pain in the bowels, constipation or diarrhea, general prostration, or even collapse. Vaughan isolated from poisonous cheese a ptomaine which he called tyrotoxicon. Vaughan's researches have been confirmed on numerous occasions.

To prevent the alteration by bacteria of milk intended to be the food of infants, the practice of sterilizing milk has been largely in vogue. Unfortunately, during sterilization the milk undergoes some kind of alteration which makes it disagree with many infants. Furthermore, among the organisms which would be likely to contaminate milk the bacilli of hay and potato, whose spores are so excessively resistant, would be prominent, and would not be sterilized by any process to which the milk intended for an infant's consumption could possibly be subjected to in the household.

The process called pasteurization is designed, not to sterilize the milk completely, but to destroy the vegetative forms of bacteria, and to destroy most pathogenic bacteria with which the milk might possibly be contaminated. The milk is subjected to a temperature of only about 75° C. This temperature is less likely to produce alteration in the milk than sterilization by steam at 100° C. According to Freeman, milk which had been pasteurized at 75° C. and distributed among the poor people in New York City, whose homes were not supplied with ice, usually kept very well even in the summer time.

It has been undertaken recently to do away as far as possible with the contamination usually inevitable in the barnyard and stable by the use of extraordinary measures to keep the cows, and especially their udders, clean; also the hands of the milker and the milk-pails; and by sprinkling the floor of the milk-room to prevent dust. The milk is to be transported to the city on ice. Milk which has been collected in this manner is furnished in several cities in the

United States under the name of "Certified Milk." The cattle from which the milk is derived are regularly inspected by veterinary surgeons as well as subjected periodically to the tuberculin test. The surroundings and drainage of the stables are investigated by physicians and sanitary engineers. The milk is also regularly analyzed by a chemist. It has been found possible to reduce the number of bacteria in milk very noticeably. As delivered in Buffalo, "Certified Milk" usually contains 15,000 to 20,000 bacteria in 1 c.c. This milk is of course sold at a considerably higher price than ordinary milk.

The number of bacteria which occurs in samples of milk varies greatly. In ordinary milk as furnished by milkmen the number of bacteria to the cubic centimeter is usually many thousands, to millions; grocer's milk may contain hundreds of thousands to millions of bacteria to the cubic centimeter; frequently figures are reached which are beyond computation.

Human milk often contains the staphylococcus epidermidis albus, and not seldom the staphylococcus pyogenes aureus, under normal conditions.

Of the different pathogenic bacteria liable to furnish a source of danger in milk, the most important is the bacillus tuberculosis. Tuberculosis is a disease to which cattle are exceedingly prone. There is good reason to believe that infants frequently acquire tuberculosis through taking as food the milk of tuberculous cows. The milk of tuberculous cows may contain tubercle bacilli when there is no tuberculous disease of the udder. The frequency of tuberculosis among milch cows sometimes becomes as high as 25 per cent., or even more. Butter derived from the milk of such cows may contain tubercle bacilli. The proper manner for the States to deal with this problem, for it is one that doubtless will fall to the individual States, has not yet been determined. The cost of killing such a large number of valuable cows would be very great. Furthermore, it is by no means certain that this procedure would eradicate the disease. The flesh of cattle also is capable of transmitting tuberculosis, but is a smaller source of danger on account of the universal practice in the United States of thoroughly cooking beef.

The rancidity of butter does not necessarily signify decomposition by bacteria, but may be a purely chemical decomposition not influenced by bacteria. It may be the result of fermentation, leading to the formation of lactic acid and other members of the fatty acid series.

"Ripening" of cream may be accomplished and hastened by the addition of bacteria to the cream. Conn has isolated an organism which generally goes by the name of bacillus No. 41, and which is said to impart a highly agreeable flavor to butter when cream has been ripened by it.

In examining milk for bacteria the number may be estimated by precisely the same technique as is used for the estimation of bacteria in water, except that the milk must be diluted; otherwise the plates are rendered opaque by the fat. It may be diluted one hundred times with sterilized water; when the number of bacteria is very great a second dilution may be required. Estimations based upon such high dilutions can only be approximate. The quantity taken for examination may be 0.1 to 1 c.c. Plates should be made immediately upon collection of the sample. If the milk stands for a few hours at the room temperature in the laboratory, the number of bacteria will become enormously increased.

The detection of a particular kind of pathogenic bacteria in milk involves very great difficulties. Staining of bacteria in milk may be done by the usual methods, but the results are not very satisfactory on account of the presence of oil.

Ahrens recommends the staining of milk with a mixture of a saturated alcoholic solution of an aniline dye with chloroform which dissolves oil; for the staining of tubercle bacilli, three to four drops of the alcoholic solution of fuchsin are added to 2 c.c. of chloroform; decolorize as usual for tubercle bacilli. The demonstration of tubercle bacilli by staining methods is likely to involve many difficulties. The procedure of injecting milk into guinea-pigs has been resorted to largely, but the results are only obtained after the lapse of weeks, when the development of tuberculosis in the guinea-pigs would indicate that the milk was tuberculous, provided that control guinea-pigs remained healthy. The most satisfactory plan will be to apply the tuberculin test to the cow from which the milk was derived.

Among the other articles of food, those are to be most carefully scrutinized which are to be eaten after little or no cooking, particularly salads, green vegetables, fruits, and the like. Under exceptional circumstances they may become agents for conveying infectious diseases. Conn showed that there was good reason for attributing an epidemic of typhoid fever among students at Middletown, Connecticut, to raw oysters. After having been collected from the oyster-beds, these oysters were placed in a small stream to fatten, where they were exposed to contamination from a sewer. Into this sewer the discharges of a case of typhoid fever were found to have been running at the time when the oysters were fattening.

The ordinary processes for curing and salting meat cannot be relied upon to destroy pathogenic bacteria.

Cases of poisoning by eating oysters, fish, meat in the form of sausage or canned meat, and other articles of food are not rare. These cases belong to the same class as those poisoned by milk and cheese already mentioned. They are due to ptomaines produced as products of bacterial decomposition.

CHAPTER V.

THE BACTERIA OF THE NORMAL HUMAN BODY.

The numerous solid tissues and organs of the human body, the fluids circulating in the interior like the blood and lymph, and the cavities that have no connection with the outer world, are entirely free from bacteria. So also the lungs, gall-bladder and ducts, urinary bladder, cervix uteri, uterus and Fallopian tubes, although having external connections, are usually sterile when in a healthy condition. When bacteria do enter the tissues from any of the surfaces their progress appears to be checked by means of the activities of the cells or fluids of the body, and if they succeed in penetrating to any considerable distance their advance is usually arrested by the nearest group of lymph-nodes, which appear to be important safeguards for preventing the dissemination of bacteria throughout the body. As a rule, the secretions of the mucous membranes are inimical to bacteria.

The skin, as might be expected, is liable to have upon it numerous bacteria, especially micrococci, and moulds. The staphylococcus pyogenes aureus, the streptococcus pyogenes, the bacillus pyocyaneus and the bacillus coli communis sometimes occur on the skin. According to Welch, it always contains the staphylococcus epidermidis albus, which may be a form of the staphylococcus pyogenes albus. This organism is of some importance to surgeons on account of its relation to the cleansing of the skin before operations. It seems in fact impossible, by any amount of cleaning, to dislodge all of the germs in the skin, especially those under the nails.

The bacteria of the exposed mucous membranes like the conjunctiva and the nasal cavity and the mouth cavity will naturally be very fluctuating both in quantity and quality; they will be, in fact, those which happen to fall upon the surface or to be drawn in from the external air.

In the mouth, however, there is a certain group of organisms more or less characteristic of it, many of which have not been successfully cultivated. These have been thoroughly studied by Miller, to whose works students are referred. (Miller, Microörganisms of the Mouth.)

Several species of spirilla have been discovered in the mouth and are found along the margins of the gums. The leptothrix buccalis, and related organisms which have a long, ribbon-like form, also occur in the mouth. The micrococcus lanceolatus (or pneumococcus) appears to be present in many human mouths. In 15 to 20 per cent. of human mouths this organism is sufficiently virulent to produce fatal septicemia when inoculated into susceptible animals. Pyogenic bacteria, especially streptococci, occur frequently, although not regularly in the mouth. According to Miller, bacteria play an important part in the production of dental caries. Certain of the bacteria of the mouth produce fermentation in the vicinity of the teeth with the formation of acids, which dissolve the calcium salts of the teeth. The softening and destruction of the decalcified matrix is then accomplished by other and liquefying forms.

The expired air coming from the mouth and nose, contrary to the popular notion, is free from bacteria, excepting those which become forcibly detached, as by efforts of sneezing and coughing.

Among the other exposed mucous surfaces, the urinary meatus and the vagina may be included. The urinary meatus and at least part of the urethra will be found to contain bacteria, which, in health, should be non-pathogenic, by different observers to contain very different species. The majority of these appear to have been introduced from the mouth in food or water. The bacillus coli communis, however, occurs invariably in health not only in the intestine of man, but also in that of many animals, especially in the lower part. The pyogenic micrococci very often occur in the intestine.

Pasteur some years ago expressed the opinion that if animals could be placed in such surroundings that bacteria could be excluded from the alimentary canal and the food, life would be impossible. This view has excited much controversy, and has apparently been disproved by the experiments of Nuttall and Thierfelder. These investigators succeeded in removing guinea-pigs from the mother by Cæsarian section, and in keeping them alive in sterile surroundings, upon sterile food, so that the contents of the alimentary canal remained sterile. In the case of ruminant animals like the cow and sheep, the decomposition of cellulose, which forms so large a part of their food, appears to be effected by bacteria. Bacteria having this power are constantly found in the stomachs of ruminants. The best known species is that called bacillus amylobacter. It is questionable whether the products of the decomposition of cellulose have any nutritive value.

CHAPTER VI.

BACTERIA IN DISEASE.

To the physician and the student of medicine the study of bacteriology is interesting chiefly on account of the great importance attributed to bacteria in producing disease. The presence in an organism of one or a number of organisms of another species, which flourish as parasites upon the first, is a phenomenon of very wide occurrence in nature. It is, in fact, nearly universal. It may be observed among plants as well as animals, for example in the familiar galls seen on some of the higher plants, and caused by an animal parasite which is harbored by the plant. We also find animals, such as tape-worms and the trichina spiralis, living as parasites upon other animals. The functions of the bacteria make them peculiarly suited to leading a parasitic existence. The fact that they possess no chlorophyll, and that they are therefore unable to form carbon compounds from the carbon dioxide of the atmosphere, makes it necessary for them to secure such compounds from preëxisting organic matter. Many of them, furthermore, flourish better when they are able to obtain their nitrogenous food from organic matter rather than from inorganic salts containing nitrogen. Most bacteria find the necessary nutriment in the dead bodies of other animals and plants; they constitute what are known as saprophytes. But some of them flourish upon the living bodies of other plants and animals in whom they may produce disease.

The phenomena of disease, we shall find, are very largely due to the numerous waste products of the activities of bacteria, which act as poisons to the host. The diseases of plants known to be caused by bacteria are not very numerous. Among them may be mentioned the pear-blight, due to micrococcus amylovorus. Among lower animals bacteria very frequently produce diseases—for example, chicken-cholera, symptomatic anthrax, erysipelas of swine, hog-cholera, tuberculosis, anthrax and glanders.

Koch formulated certain rules which he considered must be complied with in order to prove that any microörganism was the cause of a particular disease:

First. That the organism should always be found microscopically in the bodies of animals having the disease; that it should be found in that disease and no other; that it should occur in such numbers and be distributed in such a manner as to explain the lesions of the disease.

Second. That the organism should be obtained from the diseased animal and propagated in pure culture outside of the body.

Third. That the inoculation of these germs in pure cultures, which had been freed by successive transplantations from the smallest particle of matter taken from the original animal, should produce the same disease in a susceptible animal.

An *infectious disease* is a disease which is caused by a microörganism growing in the body of the animal having the disease. Such microörganisms are usually bacteria, but not always; for example, malaria is produced by a minute animal organism.

A *contagious disease* is one which is acquired from an individual having the disease. Most contagious diseases are infectious, but infectious diseases are not necessarily contagious. These words are often used very loosely, and it is no longer possible or very desirable to draw the line sharply between them. *Fomites* are the materials on which the contagious element is conveyed.

A *miasmatic disease* is a variety of infection in which the microörganisms are not received from another case of the disease, but are derived from the external world; for example, malaria.

The following is a list of the most important diseases of man caused by bacteria. The proof as required by the rules of Koch is not complete for all of them:

Tuberculosis,
Leprosy,
Glanders,
Anthrax,
Tetanus,
Malignant edema,
Bubonic plague,
Suppuration and certain inflammatory conditions allied to it,
Erysipelas,
Gonorrhea,
Lobar pneumonia,
Influenza,
Diphtheria,
Typhoid fever,
Asiatic cholera,
Relapsing fever,
Rhinoscleroma (?),
Actinomycosis.

(Malaria and amebic dysentery are caused by microscopic animal organisms).

Thrush and certain parasitic skin diseases are caused by fungi of more highly organized structure than bacteria.

In each of the following diseases there is good reason to think that the cause is some kind of microörganism, but it has not yet been discovered:

Syphilis,
Small-pox,
Chicken-pox,
Measles,
Scarlet fever,
Mumps,
Whooping-cough,
Yellow fever,
Typhus fever,
Hydrophobia.

The causes of these diseases have been very carefully sought for by ordinary bacteriological methods with indecisive results. Some of them no doubt are due to bacteria. In the case of yellow fever it is possible that the bacillus recently discovered by Sanarelli may prove to be the cause of that disease. The bacillus described by a number of observers as having been found in cases of whooping-cough may also be the cause of that disease. Bacilli have also been described in cases of measles on several occasions. It is likely that for some of the diseases mentioned other procedures than the usual methods of research will have to be devised in order that the cause may be discovered. There is reason for thinking that the protozoa may play a part in the etiology of some of them. Roux has recently announced having found in contagious pleuro-pneumonia of cattle a microbe so minute that it was barely visible with the highest powers of the microscope, so that its outlines and its morphology could not be studied. This suggests the possibility that failure to find the causes of some of the diseases above named may lie in the fact that their organisms are so small as to be nearly or entirely invisible to the microscope.

Modes of Introduction.—There are various modes in which bacteria may enter the body to produce disease. Infection of the embryo through the ovum or semen seems to be of rare occurrence. Syphilis (which may not be due to bacteria) is transmitted in this manner. The embryo may be infected through the placenta, although not commonly. The bacilli of typhoid fever and the pus-forming bacteria have been known to be conveyed through it. Tuberculosis may also be transmitted through the placenta, how frequently is still uncertain. The comparatively common occurrence of endocarditis on the right side of the heart in the fetus may be due to placental infection. Occasionally the exanthematous fevers are transmitted from the mother to the fetus.

The surfaces covered with thick stratified epithelium are not likely to be penetrated by bacteria excepting by direct introduction through some wound or other lesion. This, for instance, is true of the skin, the mouth, the vagina and bladder. The infection of bubonic plague, from which the poorer classes of Asiatics suffer in particular, appears to be introduced most often by means of wounds, especially of the feet, which are easily accounted for in these people, who habitually go barefooted. Bacteria more easily penetrate surfaces having a thin columnar epithelium such as occurs in the stomach and intestines, bronchi and bronchial tubes, uterus and Fallopian tubes.

The thin, flat epithelial cells of the air-vesicles of the lungs, as would be expected, seem to be passed with comparative ease. On epithelial surfaces covered with cilia, as in the bronchi and bronchial tubes, the uterus and Fallopian tubes, the current toward the exterior created by the cilia acts beneficially in removing bacteria.

The tonsils and lymph-follicles of the intestines, especially the lymphoid tissue of the ileum and the vermiform appendix, are points where bacterial invasion frequently begins. The lymphoid tissue of the appendix may have some influence in predisposing to infection at that point and to appendicitis. On the other hand, it is certain that the progress of many infections is checked by the lymph-nodes. That is repeatedly seen in the ordinary post-mortem wound where the spread of the inflammation along the arm is checked suddenly at the elbow or axilla. The participation of the lymphoid structures in most infections is well known. How far this is a conservative process it is impossible to say.

In most cases of infectious disease a point of entrance for the bacteria may be discovered. As a rule, the invading microbes produce a lesion at the point where they are introduced, as in the familiar cases of boils and carbuncles, when pyogenic bacteria enter the skin, or of the tubercles found in the lungs when the bacilli lodge in the respiratory tract. However, there are cases of septicemia and pyemia in which the most careful search fails to reveal the place at which the bacteria entered. The bacilli of plague usually produce no reaction at the point of entrance.

It is probable that tubercle bacilli may pass through thin epithelial surfaces and lodge in the deeper structures underneath, where they produce definite lesions. For example, they may pass by the lungs and enter the bronchial glands, and form tubercles in that situation.

In a case of malaria we find the organisms causing that disease (which, by the way, are not bacteria) in the blood, but at present our knowledge as to how they reach the blood and through what surface is defective.

Experiments on animals have shown that bacteria may be very rapidly disseminated after their introduction. The inoculation of mice, for instance, with anthrax bacilli has been known to prove fatal, although the wound was washed immediately with the strongest antiseptic solutions or the part amputated within a few minutes.

The manner in which infectious agents reach human beings varies considerably. Generally speaking, the most important element will be found to be direct or indirect connection with another case of the same disease.

Excepting under unusual circumstances, the **Air** will not contain the germs of disease. The dried pulverized sputum of cases of pulmonary tuberculosis may float in the atmosphere as dust which will contain tubercle bacilli. The pus-producing bacteria may be present in dust. Infectious elements which happen to be present in the air will usually be attached to particles of dust. Wool-sorter's disease is a name sometimes applied to anthrax in man when acquired by those engaged in the work of handling wool, and in which the anthrax bacilli or spores may be conveyed to the lungs in dust.

The atmosphere in the vicinity of cases of the exanthematous fevers at times probably contains the germs of these diseases. (The malarial organism also seems to be transmitted through the atmosphere in some manner as yet unknown.)

Water is the usual medium for the transmission of the infection in typhoid fever and Asiatic cholera. (Amebic dysentery is probably transmitted through the medium of water.)

Milk from tuberculous cows may carry the bacilli of tuberculosis; it is of most importance in the case of young infants. Typhoid fever, cholera, scarlet fever and diphtheria are sometimes conveyed through the medium of milk. Bacteria may reach the intestines in *uncooked food*, fruit and vegetables.

The **Soil** is of importance in connection with tetanus and malignant edema, whose bacteria are frequently found in soil. (The organism of malaria probably carries out a portion of its existence in the soil of damp places. Manson believes that *mosquitoes* play a prominent part in conveying the germs of malaria.)

Flies and other insects are capable of carrying the bacteria of disease, although this mode of transmission is probably unusual.

It is possible for the bacteria of disease to be derived from the individual's own body,—*auto-infection*. The microbes of lobar pneumonia, for instance, flourish in the mouths of a large number of people. The bacillus coli communis, which constantly inhabits the intestines, may invade other organs and exhibit pathogenic properties when the way is opened up for it by other disease processes.

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How much influence is to be ascribed to *individual predisposition* in contracting or warding off infection is uncertain. Welch says "the fact that some individuals are attacked, and others, apparently equally exposed to the danger of infection, escape, is not always due to any especial predisposition on the part of the former. It may be that the germs hit the one and miss the other, and we would have no more right to say that the former are especially predisposed than to say that those who fall in battle are predisposed to bullets and those who escape are bullet-proof."

Race.—The influence of racial predisposition is undeniable. For example, it is known that the negro race is much less susceptible to yellow fever than the white race.

Local Conditions often have a most important influence in determining the occurrence of infections. In endocarditis the lesion usually occurs along the line of closure of the heart-valves, indicating that the point subjected to the greatest friction is the part of the endocardium most liable to infection. Regions where there is passive hyperemia are more vulnerable, as is seen in hypostatic pneumonia. Localities which have suffered from previous inflammation or irritation are rendered more liable to subsequent infection, as when the bladder or pelvis of the kidney containing a calculus becomes the seat of a suppurative cystitis or pyelitis.

Local conditions become of great importance in surgery. The surgeon can seldom be certain of dealing with a perfectly aseptic wound, and must rely to a large extent upon the power inherent in the fluids and tissues to prevent the development of bacteria. It is important, therefore, to keep the resisting power of the tissues at the highest possible int. Injury of the tissues disposes the part to infection; do strangulation and necrosis. In operating, it is to be nembered that hyperemic and edematous parts are more likely to become infected; so also are anemic regions. An infarct of the lung which was originally sterile may be infected with bacteria through inhalation, and undergo suppuration or gangrene. The presence of foreign bodies in the tissues disposes to infection. Injection of the staphylococcus pyogenes aureus into a rabbit's tissues is not always followed by suppuration, but if a foreign body, like a piece of sterilized potato, be inserted at the same time, infection is much more likely to occur.

Variability in the Virulence of Bacteria.—The occurrence of an infectious disease depends very largely upon the virulence of the bacteria. Any species of pathogenic bacteria may vary in virulence at different times. In some cases the virulence is not easily lost, as with the anthrax bacillus; in others the virulence is maintained in cultures only with difficulty, as in the case of the micrococcus lanceolatus (of pneumonia) and the streptococcus pyogenes. As a rule, the virulence is likely to be diminished in old cultures. It may sometimes be preserved better in the ice-chest than at the room temperature. The virulence of the anthrax bacillus becomes diminished if it is cultivated at 42° C. Exposure to light and to oxygen tends to weaken the virulence; and also cultivation upon unfavorable media, such as those containing a small proportion of carbolic acid or certain other chemical germicides.

In laboratory work the virulence is usually maintained best by inoculating the bacteria from time to time into susceptible animals. Bacteria coming freshly from infected animals are likely to be highly virulent. The virulence may be increased by beginning with an especially sensitive animal like a very young guinea-pig, and progressively inoculating into less sensitive animals. The infection of relatively insusceptible animals may sometimes be produced by the injection of a very large dose of the bacteria. The addition of the toxic products of the bacteria, which may be obtained by using large doses of cultures in bouillon, makes infection more likely.

Cultivation on a particular medium may maintain or increase the virulence. This is in part the principle underlying the use of the serum medium employed by Marmorek for the cultivation of streptococci (see pages 59 and 192).

Finally, the combination of two or more kinds of bacteria may produce infection when neither one would do so alone. On the other hand, it is said that the fatal effects of an inoculation of virulent anthrax bacilli into a susceptible animal may be averted if the animal be inoculated with a culture of bacillus pyocyaneus shortly afterward.

Mixed Infection.—It is not uncommon in disease to find two kinds of bacteria associated together, producing a mixed infection. In diphtheria, very frequently, the bacillus of diphtheria is found to be accompanied in the membrane by the streptococcus pyogenes. The course of the diphtheria may be modified in this manner. The term *secondary infection* is rather loosely used. It is sometimes employed to designate an infection occurring in an individual, the resisting power of whose tissues has been weakened by some chronic organic disease. Such an infection is often called a *terminal* infection. Terminal infections are very common in cases of carcinoma, chronic nephritis, arteriosclerosis, and in many other diseases.

Concerning terminal infections Osler says: "It may seem paradoxical, but there is truth in the statement that persons rarely die of the disease with which they suffer. Secondary infections, or, as we are apt to call them in hospital work, terminal infections, carry off many of the incurable cases in the wards."

The term *secondary infection* is also used for the modification of an infectious process which has been in existence for some time, by infection with a second variety of bacteria. That takes place, for instance, in pulmonary tuberculosis, when the invasion of the already tuberculous lungs by the pyogenic micrococci leads to the formation of cavities. In this sense it will be seen that the term secondary infection is used as a name for a variety of mixed infection. In the secondary, mixed and terminal infections, the bacteria which enter secondarily are likely to be of the pus-producing varieties, especially the streptococcus pyogenes.

As to the mechanism which bacteria make use of in order to produce disease, according to our present knowledge, they work chiefly through the poisonous substances formed by them and deposited in the bodies of the persons suffering from the disease. The theory that bacteria have an important influence through the destruction of substances taken by them from the body of the patient for food, is no longer entitled to much weight; neither are we able in most cases to account for the phenomena of disease by any mechanical action on the part of the bodies of bacteria. That such action does occasionally take place may be seen in experimental anthrax in mice, where the blood-capillaries of the liver and kidneys may be completely plugged with masses of anthrax bacilli. The diseases in which the circulating blood is swarming with bacteria are much commoner in the lower animals than in man.

Toxemia.—By toxemia is meant the absorption of poisonous bacterial products from a localized point of invasion, and their dissemination throughout the body by means of the circulation. We see typical toxemias in diphtheria and tetanus. In surgery the term *sapremia* is used to cover a similar condition of affairs when the absorption proceeds from a wound or denuded surface, as may happen in the puerperal uterus.

Septicemia.—In septicemia there is not only absorption of the bacterial poisons, but the bacteria have invaded the living tissues. Bacteriologists usually employ the word septicemia to describe the wide dissemination of bacteria through the body and the presence of large numbers of them in the circulating blood. In this sense septicemias are commoner in lower animals than in man; anthrax and infection with micrococcus lanceolatus would be examples. For pyemia, see the article on Suppuration, Part IV.

CHAPTER VII.

TOXINS.

It is now generally believed that in most, if not all of the infectious diseases, the principal symptoms and lesions are to be attributed to the action of poisonous substances formed by the bacteria. The part that bacteria play can be understood best by recalling the work of the saprophytes in producing fermentation and putrefaction. It has already been shown that the poisoning that comes from eating decomposed meat, fish or cheese results from poisons which the bacteria have elaborated in the course of their growth. In infectious diseases we suppose the bacteria to grow inside of the body and to form their poisons *in it;* not before their introduction into it, as in these cases of poisoning with spoiled food. If it were possible for the cells of ordinary yeast to grow in the living human body and to produce alcohol from the grape-sugar of the body-fluids, the person so infected might be expected to suffer from alcoholic intoxication as long as the infection lasted. This *impossible* illustration may serve to make clear what does happen in an infectious disease due to bacteria, where poisons formed in a manner analogous to the formation of alcohol produce intoxications analogous to alcoholic intoxication. Certain infectious diseases exhibit the element of poisoning by bacterial products in an extremely marked manner. In tetanus the local wound may be trifling, and may seem utterly incapable of having given rise to the violent muscular spasms from which the patient suffers. In diphtheria, although the condition in the throat may be one of severe inflammation, it is, of itself, insufficient to explain the profound prostration and other symptoms of general poisoning which the case manifests.

The first bacterial poisons to be studied thoroughly were those called ptomaines. Observing the severe symptoms which follow the injection into animals of certain ptomaines derived from bacterial cultures, and having discovered the action of ptomaines in poisoning by meat, fish and the like, it was suggested that similar ptomaines, formed by the action of bacteria in the living body, might account for the symptoms of many of the infectious diseases. The ptomaines were most readily studied because of the comparative facility with which they could be isolated in a condition of purity, where their exact chemical nature could be determined.

"A *Ptomaine* is an organic chemical compound, basic in character, formed by the action of bacteria on nitrogenous matter." (VAUGHAN and NOVY.)

The tyrotoxicon of cheese- and milk-poisoning is one of the best known ptomaines. The peculiar coloring which distinguishes cultures of the bacillus pyocyaneus is due to a ptomaine called pyocyanin and its derivatives.

A group of substances of a similar nature called leucomaines have been discovered, which are formed within the body and not by the action of the bacteria. *Leucomaines* may then be defined as "basic substances which result from tissue metabolism in the body." (VAUGHAN and NOVY.)

Further study has demonstrated, however, that the symptoms of most of the infectious diseases are not due to ptomaines. Some of the poisons formed by bacteria have been described as albumens and have given rise to the name *toxalbumen.* It appears, however, that bacterial poisons are not necessarily of an albuminous nature either, and at the present time it seems best to call the bacterial poisons whose chemical nature is uncertain simply *toxins.*

Sometimes the results of the injection of excessively small doses of a toxin are so tremendous as to give color to the suggestion that the toxins may be allied to the ferments, like pepsin and trypsin, in their nature. Substances which produce effects in animals similar to the bacterial poisons may be extracted from certain plants, notably abrin, which is derived from the jequirity bean, and ricin, which comes from the castor-oil bean. The venom of poisonous snakes, which is elaborated by the epithelial cells of certain glands, also acts in much the same manner as the bacterial poisons. These poisons appear sometimes to be formed as excretions from the bacteria, and may be extracted from liquid cultures. In other cases they are apparently present in the bodies of the bacteria themselves. A substance which is highly poisonous to guinea-pigs is present in the bodies of cholera spirilla. Prudden found that the injection of dead tubercle bacilli was followed by the development of tubercles, which of course did not increase in numbers or become disseminated.

Owing to the instability of the toxins it has not been possible to isolate them in a state of purity so as to determine their exact chemical character. They have, nevertheless, been obtained in some cases in an extremely concentrated form. Brieger and Cohn obtained a toxin from tetanus bacilli of which .00000005 gram killed a mouse weighing 15 grams. Roux and Yersin obtained a toxin from diphtheria bacilli of which .00005 gram was capable of killing a guinea-pig. These figures indicate a capability for poisoning that is simply inconceivable. Such properties permit bacteria growing in a comparatively limited area to manifest their evil effects at remote parts of the body, as the guns used in modern warfare can throw an explosive projectile to work destruction at some point miles distant, and perhaps unseen.

A curious and unexplained effect of some toxins is the production of minute areas of necrosis in certain viscera, as the liver. Such "focal necroses" have been observed to be formed by the poisons of the bacilli of diphtheria, of typhoid fever, and of the micrococcus lanceolatus (of pneumonia), and following the injection of abrin and ricin.

Besides the poisonous substances produced by the bacilli of diphtheria and of tetanus, toxic substances have been obtained from the spirillum of cholera, from the bacillus of typhoid fever, and from the bacilli of tuberculosis and glanders. The extract from cultures of tubercle bacilli, called tuberculin, and that from glanders bacilli, called mallein, contain toxins produced by these germs, and will be spoken of in connection with the bacteria themselves. There is good reason on both clinical and experimental grounds to believe that toxic substances are formed by the micrococcus lanceolatus (of pneumonia). Substances have been obtained from the pyogenic bacteria, injection of which produces suppuration. The symptoms of a disease as it occurs in man cannot be imitated in any other case as accurately as happens after the injection of the toxins of tetanus and diphtheria in the lower animals.

CHAPTER VIII.

IMMUNITY.

Certain facts relating to immunity, some of which were observed many years ago, are extremely interesting, but very difficult of explanation. Even in the light of recent bacteriological researches their interpretation is by no means clear. The immunity which an individual who has suffered from an attack of measles or scarlet fever possesses against a second attack of the same disease is well known; so also is the immunity against small-pox which is conferred by vaccination. Such an immunity is called "acquired." There is also a "natural" immunity. Although sheep, as a rule, are easily infected with anthrax, Algerian sheep are not likely to be infected. Field-mice are susceptible to glanders and house-mice are not. House-mice are susceptible to mouse-septicemia and field-mice are not. The immunity which belongs to a race, but not to a whole species, is sometimes called "racial." The occurrence of immunity against a second attack of an infectious disease has given rise to numerous hypotheses and has stimulated much experiment. One theory supposes that after an attack of the disease certain bacterial products are retained within the body which prevent a second invasion. Another theory supposes that the attack of the disease exhausts the supply of some substance necessary for the growth of the microbes.

Metschnikoff has described under the name "phagocytosis" a phenomenon which, he maintains, explains immunity and recovery from bacterial invasion. This theory supposes that certain cells of the body which possess ameboid movement, chiefly polynuclear leucocytes, grasp the invading bacteria, swallow them up into their own protoplasm, and destroy them by their digestive functions. Metschnikoff states that he has observed this process many times. Other investigators have also seen bacteria enclosed within the bodies of leucocytes. It is urged by some that it is more likely that the bacteria are already dead when the leucocytes devour them. It is well known that a suppurating part contains large numbers of leucocytes, and one of the most characteristic events in the inflammatory process is the migration of leucocytes to the point of injury. This indicates a positive chemotaxis for leucocytes on the part of substances in the inflamed area. Metschnikoff believes that the function of the leucocytes thus drawn to the point where the bacteria of suppuration have entered is to destroy these bacteria and to arrest their further progress. On this theory the leucocytes or phagocytes are sometimes called the policemen of the body.

In certain infectious diseases the number of leucocytes, chiefly of the polynuclear neutrophile variety, in the circulating blood is increased (leucocytosis). This is the case usually in lobar pneumonia and acute suppurative infections. In other infectious diseases there is no leucocytosis; for example, tuberculosis, typhoid fever, and malaria.

At the present time investigators seem inclined to attribute the resisting power of the body against microörganisms and their products to the fluids of the body. Nuttall made the important discovery that the serum of the blood deprived of all cells possesses the power of destroying pathogenic bacteria.

The ingredients of the blood-serum that exert the bactericidal influence have been named *alexins;* they have also been called defensive proteids. The nature of these substances has not been determined with certainty. Vaughan believes them to be nucleins. Such substances apparently serve as safeguards to the body against all kinds of bacterial invasion. They have not necessarily a specific action as regards any particular kind of infection.

It appears, however, that recovery from certain infectious diseases is accompanied by the formation of substances which protect the individual against that particular disease and no other. It has been found possible, at all events, to bring about the recovery of animals infected with diphtheria or tetanus by the injection of the blood-serum derived from other animals which had recovered from the disease.

The use by Jenner of the virus obtained from the disease of cattle known as "cow-pox" as a preventive against small-pox in man was the first important attempt to produce an artificial immunity. The procedure was, from the first, empirical, and to this day we are ignorant as to the manner of its action.

Pasteur conceived the idea of attenuating the virulence of the microbes of fowl-cholera by prolonged exposure to oxygen, and of making use of the attenuated virus as a vaccine against the disease. The same principle was shortly afterward applied by him to the preparation of a vaccine against anthrax. Anthrax bacilli were cultivated at a temperature of 42° C., at which they do not produce spores, and were freely exposed to the atmosphere. In this manner Pasteur obtained a variety of bacilli of so little virulence as not to produce death when inoculated into animals that were ordinarily susceptible. Yet animals that had been vaccinated with this virus were able afterward to resist inoculation with fully virulent anthrax bacilli. Other methods of diminishing the virulence of pathogenic bacteria have also been made use of (see page 134).

Immunity has sometimes been produced in the lower animals by inoculation with very minute amounts of fully virulent cultures of bacteria. It has been found, furthermore, that the injection of the bacterial products obtained from cultures, but entirely free of the bacteria themselves, in doses insufficient to produce death, is, in some instances, perfectly effectual in producing immunity. Ehrlich discovered that the vegetable toxins, abrin and ricin, behave in a manner very similar to the bacterial poisons when injected into animals, and that by their injection an immunity for the same poisons may be secured. There is an analogy between the tolerance acquired in this manner for bacterial and other toxins and that which victims of the morphine and cocaine habits have for immense doses of those drugs.

Besides the diseases which are confined to the lower animals, by one expedient or another it has been found possible to secure experimentally in animals immunity against infection with anthrax, typhoid fever, Asiatic cholera, diphtheria, tetanus, the micrococcus lanceolatus (of pneumonia), bacillus pyocyaneus, proteus vulgaris, bacilli coli communis, the pyogenic micrococci and hydrophobia. It is to be understood that in most cases the infection in the lower animal against which it is protected is a toxemia or septicemia, and not the counterpart of the disease as it occurs in man.

Ehrlich found that the milk of animals which had been immunized against abrin and ricin conferred immunity upon young animals. In most cases we look to the blood-serum for the immunizing agent.

It is customary to call the unknown bodies present in the fluids of immunized animals, and to which their immunity is due, by the name of "antitoxins." The nature of these substances is unknown. They must be formed through the activities of the cells of the body, as a result of what we may call a *reaction* against the bacterial poisons. As there is some reason for thinking the leucocytes may take a prominent part in the preparation of such substances, it is possible that a compromise may come about between the phagocytic theory of Metschnikoff and the purely "humoral" theory, which attributes to the fluids of the body all the phenomena of immunity and resistance to bacterial invasion. It has been suggested that the action of antitoxins is chemical, and that they neutralize the toxins as an alkali neutralizes an acid. It is possible that the antitoxin neutralizes the effect of the toxin upon the cells of the body after the manner of what is called a "physiological antidote," as we suppose atropine to antagonize the effects of morphine.

The kind of immunity which results from the injection of blood-serum or other substances derived from an immune animal, and supposed to contain an antitoxin, is called "*passive immunity.*" "*Active immunity*" results from an ordinary attack of an infectious disease, or from an attack excited artificially by introducing small doses of virulent cultures or large doses of attenuated cultures, or by the injection of bacterial products freed from the bacteria themselves. Active immunity is usually more enduring than passive immunity, but passive immunity, established through the direct introduction of antitoxins, may be brought about more quickly than would be possible for an active immunity.

It appears in some cases that when animals have been made immune to an organism, the organism becomes disintegrated when mixed with the animal's serum. This has been demonstrated by Pfeiffer for infection with the spirillum of cholera. The reaction does not take place outside of the body of the immune animal, but goes on rapidly in the peritoneal cavity. Since no such disintegration takes place when other bacteria than the spirilla of cholera are injected into the animal made immune against cholera, it has been suggested by Pfeiffer that this reaction could be made use of in the diagnosis of that disease. Although the serum of the immune animal fails to exercise its disintegrating effect in a test-tube, yet if a few drops of it be introduced along with the cholera spirilla into the peritoneal cavity of an animal not immune, the same disintegration takes place. Animals that have not themselves been immunized may be protected against infection by sufficient doses of serum from an immunized animal.

Quite recently it has been shown that the blood-serum of patients having typhoid fever contains a substance which, when mixed with living typhoid bacilli, causes them to gather into groups or clumps, and at the same time to lose their motility. In the great majority of cases no such clumping occurs when the blood of typhoid cases is mixed with other bacteria than the bacilli of typhoid fever. The nature of this agglutinating substance, as it is called, is not known, nor is its significance understood. It has been applied to the diagnosis of typhoid fever, where it is called the "Widal reaction," and will be discussed in connection with the bacilli of typhoid fever. Similar agglutinating bodies form in many other infections—notably with the cholera spirillum, bacillus pyocyaneus, bacillus proteus, and bacillus coli communis—but they have not yet been thoroughly studied.

In the treatment of disease in man the successful application of the principles which have been described as underlying the formation of antitoxins has been confined to hydrophobia, tetanus and diphtheria. Vaccination against small-pox may depend upon similar principles.

Inoculation against Hydrophobia.—Pasteur discovered that rabbits were susceptible to hydrophobia, when portions of the medulla oblongata of a dog which had died of the disease were placed under the dura mater of the rabbit. Spinal cords taken from rabbits thus infected are placed in a desiccating chamber. Under these circumstances the unknown virus is supposed to undergo diminution in virulence. Emulsions are made from spinal cords desiccated in this manner. Beginning with a spinal cord which has been desiccated a longer time, and in which the virulence of the poison is supposed to have been much reduced, injections are made at intervals from cords that have been subjected to desiccation for shorter and shorter periods, and therefore of greater and greater virulence. At the end of about the twenty-fifth day the patient is supposed to be immune against hydrophobia. Although the injection takes place after the patient has been bitten by a rabid dog, owing to the long incubation period that is generally observed in hydrophobia—usually one to two months—it is hoped that the patient may be rendered immune against the disease before the period of incubation has ended. Reports of cases managed according to this method have been conflicting, but, on the whole, the weight of evidence seems favorable to the treatment.

Diphtheria Antitoxin.—It is first necessary to obtain the toxin formed by diphtheria bacilli in a concentrated form. Diphtheria bacilli are cultivated in alkaline bouillon, in flasks plugged with cotton, exposing a large surface to the air. The cultures are grown in the incubator. After three or four weeks they are ready, and are filtered through unglazed porcelain. The filtrate contains the toxin. The animal usually employed is the horse; the dog, the sheep and the goat have also been used. The horse should be healthy, and the presence of tuberculosis and glanders should have been excluded, testing with tuberculin and mallein. The toxin is injected into the horse in small doses—about 1 c.c. of the filtrate from the bouillon culture. The injection is repeated at intervals, using larger and larger doses, until the animal is able to tolerate a very large dose indeed—as high as 300 c.c. If the treatment is successful the general condition of the animal should not suffer. The injections last over a long period—several months. The general condition of the animal remaining good, the resistance to these large doses of toxin is presumed to indicate the formation of a concentrated antitoxic substance in the blood. Through an incision in the skin a trochar is inserted into the jugular vein; the blood is conducted into sterilized flasks with every precaution to insure sterility. The blood is allowed to coagulate on ice, and the serum is withdrawn with sterilized pipettes. This serum is the so-called antitoxin used in medical practice.

The invention of the procedure in substantially this form we owe to Behring, who devised a standard to express the potency of the serum, called an "immunizing unit." The immunizing unit, according to Behring, is 1 c.c. of serum, of which 0.1 c.c. injected into a guinea-pig will protect against the injection of a dose of toxin ten times large enough to kill the animal if the toxin were injected alone. It has been found possible to procure an antitoxin with a high degree of concentration, so that 500 to 1500 of Behring's immunizing units may be contained in a quantity of the serum which it is practicable to give at a single hypodermic injection. The large volume of statistics that have been collected from hospitals and from physicians in private practice indicates that the use of this serum has effected a very great reduction in the mortality from diphtheria.

The injection into animals artificially infected with tetanus of a remedy prepared according to similar principles for the cure of tetanus has produced very gratifying results. The value of the *tetanus antitoxin* as a therapeutic agent for tetanus in man is by no means certain. It may be said to be on trial at the present time.

CHAPTER IX.

DISINFECTANTS AND ANTISEPTICS.*

A **disinfectant** or **germicide** is a substance capable of killing bacteria. The latter term is of more recent development than the former, and apparently needed on account of the loose application of the term disinfectant.

An **antiseptic** is a substance capable of preventing the growth and reproduction of bacteria. It differs from a disinfectant or germicide in that it simply prevents development without actually killing.

A **deodorizer** is a substance capable of so changing a noxious odor that it is less unpleasant to the sense of smell. At the present time the term is usually and properly restricted to those substances which, without disinfectant action, simply replace or destroy an odor.

TESTING ANTISEPTICS AND DISINFECTANTS.

The determination of the antiseptic value of a material is a comparatively simple matter. A virulent culture of the organism used as a test is inoculated into sterile bouillon containing a known quantity of the antiseptic. The process is repeated with varying strengths of the material until the smallest quantity of it capable of preventing growth is determined. This dilution may be considered the antiseptic value of the material in question for the organism used, under the conditions of the test.

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Determination of the disinfectant power of a substance is a problem of much greater magnitude, and the method used must be altered more or less to suit the properties of the substance tested. It is obvious that some of the substance tested remains in contact with the organisms in the method given for determining the antiseptic value, and that we do not know whether the bacteria are alive and merely inhibited in growth, or actually killed.

Sternberg's Method.—To a measured quantity of a virulent bouillon-culture of the test-organism is added a known quantity of the substance to be tested. After varying lengths of time inoculations are made from this mixture into culture-media, preferably bouillon, and growth watched for under suitable conditions as to temperature and the like. The shortest exposure of the test-organism to the smallest quantity of the substance is taken as the germicidal value of that substance for the particular organism used.

Koch's Method.—Usually employed for spore-bearing organisms like anthrax. Small pieces of sterile silk or cotton thread are soaked for some hours in a bouillon-culture of the test-organisms. They are removed, partially dried, and then placed in a solution of known strength of the substance being tested, and exposed for a definite length of time. The thread is removed from the solution, washed carefully in sterile water, planted in bouillon, and growth is watched for. As in other methods, the greatest dilution of the germicide that will kill the test-organism in the shortest time is taken as the germicidal value of that substance for the organism used.

Both these methods are open to serious sources of error, particularly in the testing of powerful germicides. In Sternberg's method, small quantities of the substances tested may be carried over with the organisms, and, if a powerful germicide, in sufficient amount to prevent growth, and thus give erroneous results. In Koch's method this factor is partially obviated by washing in sterile water after exposure to the germicide. This does not remove another source of error, namely, the chemical action that may take place between the substance and the protoplasmic contents of the bacterial cell. This action may extend deeply enough to restrain the growth of an organism for a very long time without actually killing it. When placed under suitable conditions, such union may be broken up and the organism regain its power to develop. It has been suggested that, to remove errors in the above methods, test-cultures containing bacteria supposed to be killed by the smallest quantity of germicide be inoculated into susceptible animals; but Sternberg's experiments in this direction have shown that bacteria may become so altered in virulence by the action of germicides insufficient to kill, that animal inoculation experiments are worthless.

Geppert suggested a valuable modification of these methods while determining the germicidal value of bichloride of mercury. After exposing his test-organism to bichloride of mercury, and before inoculating into bouillon to determine death of the organism, he treated with a dilute ammonium sulphide solution, thus effectually neutralizing any mercury-salt remaining.

Sedgwick developed this method still further, and to him we are indebted for demonstrating its accuracy and practicability.

Method.—To 15 c.c. of sterile water in a 60 c.c. Erlenmeyer flask add 2 c.c. of a virulent culture of the test-organism. Then add a solution of the substance under investigation in the proportion necessary to give the dilution wished. Mix thoroughly, and allow this "action-flask" to stand as long as it is desired to have the germicide in contact with the test-organism (action-period). Transfer 0.5 c.c. from the action-flask to a flask containing 200 c.c. of a solution of some chemical capable of decomposing the substance being tested with the formation of inert or insoluble compounds. In this "inhibition-flask" the strength of the solution should be such that molecular proportions of the chemical are present in sufficient quantity to combine with all the germicide carried over. The inhibition-flask is shaken for 30 seconds, and 1 c.c. transferred from it to 100 c.c. of sterile water in another, the "dilution-flask." After two minutes, three agar tubes are inoculated with 1 c.c. each from the dilution-flask, plated, and growth watched for.

Control-experiments should be performed to determine that the dilution of the test-culture is not too great when carried through the three flasks. It likewise should be determined that the inhibiting chemical has no effect on the bacteria.

What the inhibiting chemical shall be must be determined for each individual case. For salts of the heavy metals ammonium sulphide answers well; for mercury salts, stannous chloride may be used; for formaldehyde, ammonium hydrate; for carbolic acid, sodium sulphate.

The testing of gaseous disinfectants, such as sulphur dioxide and formaldehyde, must be conducted under conditions as nearly parallel to actual practice as possible. The test-organisms may be exposed on threads, after Koch's method, and acted upon by a known volume strength of disinfectant for a known length of time. Subsequent treatment of the organisms with a suitable inhibitor is necessary when possible, and should growth occur in the cultures following, the test-organism should be recognized in order that possible contamination by extraneous organisms may be excluded.

In determining the value of germicides for sterilizing ligatures, the student can apply methods based on the foregoing principles. Great care and ingenuity are necessary to arrive at correct conclusions, particularly in the case of animal tendons. In many instances quite stable compounds are formed between tendon and germicide, and living organisms may be so imbedded in such a substance that subsequent growth in a test-culture is impossible. The use of a suitable inhibitor, and, prior to final culture-tests, a prolonged soaking in sterile water, will promote the accuracy of the results.

CHEMICAL DISINFECTION.

Heat properly applied is the simplest and at the same time the surest disinfectant; but for many purposes it cannot be used, and we have recourse to those chemicals that practice and investigation have shown to be of value. An immense number of substances possess germicidal properties, but unfortunately, the majority are objectionable in that they are expensive, intensely poisonous, or so corrosive that damage may be done to articles of value with which they may come in contact.

In the following pages only those substances which are in common use or seem to be of special value will be considered.

Mercuric Chloride or Bichloride of Mercury.—This substance is probably more commonly used than any other one disinfectant. In the strength of 1–1000 it will sometimes kill the spores of anthrax within a few minutes (see Bacillus anthracis, Part IV.). It is claimed that its affinity for albuminous bodies, and the readiness with which it combines with such substances, detracts from its value for some purposes. On the other hand, many observers claim that the albuminous combinations formed under such circumstances are soluble in an excess of albuminous fluid, and that its value as a germicide is not affected thereby.

To obviate this possible difficulty it is customary in practice to combine the bichloride of mercury with some substance that will prevent the precipitation of the mercury salt by albumen. For this purpose 5 parts of any one of the following substances to 1 part of bichloride of mercury may be used—hydrochloric acid, tartaric acid, sodium chloride, potassium chloride or ammonium chloride. A very practical stock-solution for laboratory purposes has the following composition:

Hydrochloric acid,	100 c.c.
Bichloride of mercury,	20 grams.

5 c.c. in a liter of water make a solution of about 1–1000 strength.

Mercuric Iodide.—An extremely high antiseptic value has been placed on this substance by Miquel, who claims that the most resistant spores are prevented from developing in a culture-medium containing 1–40,000. In combination, as potassio-mercuric iodide, it has been used in soaps (McClintock) with very favorable results. The substance is not extensively employed, and further investigation is necessary to determine its true value.

Silver Nitrate.—This salt probably occupies the next position to the bichloride of mercury in disinfectant power. Behring claims it to be superior to bichloride of mercury in albuminous fluids. The anthrax bacillus is killed by a solution of 1–20,000 after two hours' exposure. At least forty-eight hours' exposure to a 1–10,000 solution is required to kill the spores of anthrax. It is very irritating, and possesses strong affinities for chlorides, forming with them insoluble chloride of silver, a salt without germicidal value. For these reasons the use of silver nitrate is limited. In the solutions usually employed for douching the cavities of the body, the available silver nitrate is immediately converted into the insoluble chloride, and little if any germicidal action takes place. To this fact may be ascribed the varying clinical results reported.

Many semi-proprietary silver compounds are on the market, introduced to replace the nitrate and its objectionable features. The most important are argentamin, argonin and protargol, all organic silver combinations. They do not combine with chlorides, are less irritating than the nitrate, and, not coagulating albumen, they possess greater penetrating power. Clinical reports and investigations have been so contradictory thus far that their value cannot be readily estimated.

Carbolic Acid.—One of the most important and most widely-used disinfectants. It is usually employed in strengths of from 1 to 5 per cent. A 3 per cent. solution will sometimes kill the spores of anthrax after two days' exposure (see page 211). In the absence of spores the anthrax bacillus is destroyed by a 1 per cent. solution in one hour. The less resistant pus cocci are destroyed rapidly by a 2 per cent. solution. Combination with an equal proportion of hydrochloric acid enhances the efficacy of carbolic acid to a marked extent. This is due to the prevention of albuminous combinations, thus allowing greater penetration of the disinfectant.

Many other substances closely related to carbolic acid are used and possess marked germicidal properties. Among them may be mentioned creolin, cresol and lysol. They are all slightly superior to carbolic acid in actual germicidal value.

Aniline Dyes.—Many of these substances possess germicidal properties, notably pyoktanin (methyl-violet). A solution of 1–5000 will kill the anthrax bacillus in two hours. A much stronger solution, 1–150, is required to kill the typhoid bacillus in the same time. Malachite-green is said to possess even greater germicidal value than pyoktanin. Methylene-blue also possesses considerable germicidal power.

Formaldehyde.—A gaseous substance placed on the market in a 40 per cent. aqueous solution. Remarkable claims have been made for this substance, and numerous investigations have shown it to possess, both in the liquid and gaseous forms, wonderful disinfecting power under certain conditions. It is a noticeable fact that the more recent the investigation the lower the value placed upon it. In solutions of 1–1000 an exposure of twenty-four hours is necessary to destroy the staphylococcus pyogenes aureus, while 1–5000 is sufficient to restrain its growth (Slater and Rideal). Its use in a gaseous form as a house-disinfectant is by far the most important application at the present time.

Harrington's recent investigations have shown that an atmosphere produced by vaporizing 435 c.c. of formalin (40 per cent. aqueous solution of the gas) in 1000 cubic feet of air space, equivalent to 1 quart to a room 15 feet square and 10 feet high, will destroy all exposed organisms in half an hour; when protected by one fold of cotton-cloth, an exposure of one and one-half hour is necessary. In a perfectly dry atmosphere the gas penetrates slightly, and will disinfect through one layer of cotton-cloth; in a moist atmosphere no penetration can be obtained.

In vaporizing the gas many methods have been employed. Simple evaporation of solutions with or without heat cannot be relied upon. With heat, the process is wasteful, and a solid, polymerized compound may be formed in and on the edge of the pan. It has therefore been found necessary to use special forms of lamps or generators for its production, a few of which are mentioned below.

Sanitary Construction Company's Lamp.—This lamp consists of a tank to hold the formaldehyde solution, and a spiral tube by which the solution is slowly conducted through a flame and vaporized. The necessary amount of solution is placed in the tank and the apparatus started, outside the room, the gas being conducted through the keyhole by a suitable tube.

Trillat Autoclave.—A small silver-lined pressure-boiler, fitted with lamp, safety-valve, pressure-gauge, thermometer and escapement-tube. The necessary amount of formaldehyde solution is placed within the apparatus, together with an equal amount of a 20 per cent. solution of calcium chloride; the addition of the latter salt is to prevent formation of the solid polymeric modification, the so-called paraform. The autoclave is closed and heated from below to a temperature of 135° C. The escapement-valve is then opened carefully and the gas allowed to enter the room slowly through the escapement-tube, which has meanwhile been passed through the keyhole. About thirty minutes are required to discharge all the gas from 500 c.c. of solution. If the temperature has not been allowed to go above 135° C. the gas will contain but little moisture and possess its maximum efficiency.

Schering Lamp.—This lamp is intended to utilize paraform or para-formaldehyde, a polymeric modification of formaldehyde, occurring as a white salt. It is decomposable by heat, yielding formaldehyde gas. It is placed on the market in the form of tablets, each one of which yields a definite amount of gas. The lamp consists of a small iron tray for the reception of tablets, and so arranged above the heating-apparatus that sufficient draught is created to carry off the gas as rapidly as formed. In operating, a sufficient number of tablets are placed on the tray, the lamp lighted and placed in the room to be disinfected.

Methyl-Alcohol Lamps.—Several of these lamps are on the market, all operating on the well-known principle of the oxidation of wood-alcohol to formaldehyde when the alcohol is vaporized by projection against a heated, platinized, asbestos disk. In operating such an apparatus, the alcohol is lighted until the asbestos disk becomes hot. The flame is then extinguished; the heat from the disk is sufficient to vaporize the alcohol, which undergoes oxidation and keeps the disk at a red heat. When the apparatus is operating in a satisfactory manner the room is closed and disinfection allowed to proceed.

Sulphur Dioxide.—This substance is used extensively for house disinfection, and is usually prepared by burning sulphur. Much difference of opinion exists regarding the value of it as a disinfectant. The spores of anthrax are not killed by several days' exposure to the liquefied gas. Anthrax and other bacilli are destroyed in thirty minutes when exposed on moist threads in an atmosphere containing one volume per centum of the gas. An exposure of twenty-four hours in an atmosphere containing four volumes per centum of the gas will destroy the organisms of typhoid fever, diphtheria, cholera and tuberculosis. The presence of moisture greatly enhances the activity of the disinfectant, owing to the formation of the more active sulphurous acid.

In practice, at least 3 pounds of sulphur per 1000 cubic feet should be used, and moisture must be present. This latter requirement can be fulfilled by evaporating a few quarts of water within the tightly-closed room just prior to generating the gas. In using powdered or flowers of sulphur, the necessary amount is placed on a bed of sand or ashes in an iron pot, which should rest on a couple of bricks in a pan or other vessel containing an inch or two of water. The sulphur is ignited by means of some glowing coals, or by moistening with alcohol and applying a match. Difficulty is often experienced in keeping the sulphur burning, and for this reason it is surer and more convenient to use the so-called sulphur candles now on the market. In operating with these, a sufficient number are placed on bricks in a pan of water and the wicks lighted. Liquefied sulphur dioxide may be used, and can now be obtained in convenient tin receptacles containing a sufficient quantity for the disinfection of an ordinary room. The can is opened by cutting through a soft metal tube projecting from the top. The fluid vaporizes at the room temperature, and it is simply necessary to place the can in a convenient porcelain dish and allow the fluid to evaporate.

Sulphur dioxide is objectionable on account of its lack of power when dry, and on account of its corrosive action on metal and its bleaching effect on hangings and draperies in the presence of moisture; it is, therefore, preferable to use formaldehyde when possible.

Chlorine.—A very active gaseous disinfectant, particularly in the presence of moisture. An atmosphere containing 1 per cent. of the dry gas is fatal to anthrax spores in three hours. The anthrax bacillus is killed in twenty-four hours by exposure to a moist atmosphere containing the gas in the proportion of 1–2500. The bacillus of tuberculosis is killed by an exposure of one hour to a moist atmosphere containing the gas in the proportion of 1–200. Extremely minute quantities in solution will prevent the development of putrefactive organisms. The substance has been used for house and ship disinfection, but is now seldom employed on account of its extremely irritating properties and the difficulty of handling it.

Bromine.—Used in the gaseous and liquid form. The dry vapor possesses but little disinfectant power; when moist it is much more efficient. In saturated aqueous solution it will kill the anthrax bacillus in twenty four hours.

Calcium Hypochlorite.—A most practical and valuable disinfectant, depending for its efficiency on the available chlorine contained in it. Its alkalinity favors penetration, and for many purposes it cannot be excelled. A 1 per cent. solution will destroy anthrax spores in one hour. A solution of the same strength will disinfect typhoid stools in ten minutes.

Lime.—The addition of 0.1 per cent. of unslaked lime to fluid-cultures of the typhoid bacillus and cholera spirillum will render them sterile in four or five hours. Typhoid dejecta are sterilized in six hours by the addition of 3 per cent. of slaked lime; the addition of 6 per cent. will accomplish the same result in two hours. A convenient form for practical use is an aqueous mixture containing 20 per cent. of lime—so-called milk of lime. Typhoid and cholera dejecta are sterilized in one hour after the addition of 2 per cent. of this mixture. From the foregoing facts it would seem probable that lime or whitewash as ordinarily applied would possess disinfectant properties. Experimental work has demonstrated this to be a fact. The organisms of anthrax, glanders and the pus cocci were destroyed within twenty-four hours by one application. For spore-forming organisms and the bacillus of tuberculosis the power is not so great, the latter organism not being destroyed by three applications of the whitewash. This is due, perhaps, to the large amount of fatty matter in the bacillus of tuberculosis, and suggests the possibility of enhancing the efficacy of the lime by the addition of a small proportion of caustic alkali.

Hydrogen Peroxide.—This substance is placed on the market in solutions varying in strength from 10 to 30 volumes; the mode of expression indicating that corresponding solutions will liberate ten to thirty times their volume of oxygen when appropriately treated. The addition of 1–1000 to a water infected with the typhoid bacillus and the cholera spirillum will render it sterile in twenty-four hours. Van Tromp asserts that the addition of 1–10,000 to a polluted water will render it sterile in twenty-four hours. It possesses the property of rapidly oxidizing purulent secretions, and on this account is much used for cleansing infected wounds. It deteriorates in strength so rapidly that only fresh solutions of known strength should be used.

Potassium Permanganate.—Koch asserts that a 3 per cent. solution will destroy anthrax spores in twenty-four hours, but that a 1 per cent. solution cannot be depended upon to kill pathogenic organisms. Its disinfectant value in practice is very low on account of its ready decomposition by inert material. In the dilute solutions usually used for medicinal injections and irrigations no disinfectant action occurs.

Iodoform.—This substance possesses little if any disinfectant power. It is mildly antiseptic in moist wounds, due to the gradual liberation of small quantities of iodine.

Boric Acid.—This material possesses practically no disinfectant power. It is a mild antiseptic when applied as an undiluted powder to wounds. A saturated aqueous solution is much used, and is weakly antiseptic.

Essential Oils.—Many of these bodies possess germicidal value, notably the oils of cinnamon and cloves. The oil of mustard is also a valuable disinfectant, but so irritating that the pure oil cannot be used. The use of powdered mustard in the autopsy-room will remove the foul odor from the hands more rapidly and completely than any other means.

Ferrous Sulphate (*Copperas*).—This salt has been much used, but possesses only feeble disinfectant powers. A 3 per cent. solution requires three days to kill the bacillus of typhoid fever. On account of its affinity for ammonia and sulphides it is an efficient deodorizer for temporary use, but cannot be relied upon to kill the bacteria producing the noxious gases.

Cupric Sulphate (*Blue vitriol*).—This salt is quite an efficient disinfectant. In a solution of 1–3000 the spirillum of cholera is destroyed in ten minutes. A 5 per cent. solution will kill the typhoid bacillus in ten minutes. A solution of from 2 to 3 per cent. in strength can be relied upon to destroy all pathogenic organisms that do not form spores.

Zinc Sulphate.—This salt is a very feeble disinfectant. Pus cocci are not destroyed in two hours by a 20 per cent. solution. As a deodorizer it has about the same value and acts in the same way as ferrous sulphate.

Zinc Chloride.—A 2 per cent. solution will kill pus cocci after an exposure of two hours. It is therefore a much more powerful disinfectant than the sulphate.

Disinfection of Dejecta and Urine.—A 4 per cent. solution of calcic hypochlorite is most efficient and rapid for this purpose. A convenient solution contains 6 ounces of the salt to 1 gallon of water. The excreta should be received in a suitable vessel and immediately mixed with an equal bulk of the disinfectant. The contents of the vessel should be allowed to stand for one hour before emptying. A 20 per cent. milk of lime is just as efficient, and possesses the advantage of cleanliness and lack of odor. It should be used in the same quantity and allowed to act for the same length of time. A 5 per cent. solution of carbolic acid may be used, but should be allowed to act for at least four hours.

Disinfection of Sputum.—The chemical disinfection of tuberculous sputum is somewhat difficult on account of the large amount of albumen in it and the fatty matter associated with the bacillus of tuberculosis. Dilute solutions of bichloride of mercury are apt to be decomposed and rendered inert by the albumen. Carbolic acid is open to the same objection, but its combination with hydrochloric acid can be used successfully in a strength of 5 per cent. each. Milk of lime cannot be relied upon for this purpose. A 4 per cent. solution of calcic hypochlorite is the best for general use, and the spit-cup should he kept nearly full of this solution.

Disinfection after Post-mortems.—After autopsies on infectious cases it is necessary to disinfect the table and fluid-products coming from it prior to emptying into the sewer. The table may be successfully disinfected by a liberal sprinkling with 4 per cent. calcic hypochlorite solution. All fluids should be treated with an equal quantity of the same solution. The table should not be cleaned for at least one hour after application of the disinfectant. The same rule applies to the disinfection of the fluids—an exposure of at least one hour to the disinfectant before final disposition.

House Disinfection.—After infectious disease it is essential that the house or the apartment in which the patient has been confined should be disinfected. It is rarely necessary to carry out the process in more than two rooms; but should it be so, the process can be applied to the whole house.

After thorough bathing of the patient, preferably with an antiseptic soap, the individual should be wrapped in a clean sheet and removed to a clean room. All articles or materials that are of little value should be destroyed. All bedding, towels and the like should be placed in wooden tubs and covered with a 1–1000 solution of bichloride of mercury. The room should then be made as nearly air-tight as possible; this can be accomplished by pasting strips of paper over registers, cracks, space between window-sashes and the like. Formaldehyde gas is then passed through the keyhole into the room in sufficient quantity to destroy the infectious element. The room should be sealed for at least twelve hours, after which time it may be opened and aired. The process is completed by washing all exposed surfaces in the room with 1–1000 bichloride of mercury. This latter requirement is not essential if the gaseous disinfection has been complete, but since we have no absolute knowledge on this point, the secondary washing should be carried out. This method can be considered reliable for surface disinfection, but for the interior of mattresses and stuffed furniture-cushions it is not certain. In all cases where absolute disinfection is demanded, such articles must be ripped apart and loosely exposed to the gas. They may be disposed of by fire or sterilized by steam under pressure. The latter method must necessarily be a matter of municipal control, and can only be carried out by means of suitable apparatus in the hands of a municipal disinfecting corps. Instead of formaldehyde, sulphur dioxide may be used for room disinfection, but in the light of present knowledge the formaldehyde method is superior.

CHAPTER X.

THE PREPARATION OF INSTRUMENTS, LIGATURES, DRESSINGS, ETC., FOR SURGICAL PURPOSES.*

THE following formulæ have been selected from the many published, for the reason that they are used by the best surgeons and in the best hospitals in this country to-day:

Disinfection of the Hands.—It should be remembered that absolute sterilization of the skin is often impossible, as organisms can be cultivated from it after the most careful attempts at disinfection; but even if complete sterilization is not accomplished, it can be shown that the organisms of the skin may be so far inhibited as to be incapable of further growth. Should such organisms reach a wound, they are also antagonized by the resisting powers of the tissues (see pages 120 and 142).

The organisms usually found on the skin are the ordinary pyogenic bacteria, the staphylococcus pyogenes aureus, citreus and albus and the streptococcus pyogenes, and the staphylococcus epidermidis albus, whose normal habitat is the skin, and which is the most common cause for stitch abscesses. Amongst the rarer forms which may be present are the bacilli of tetanus, of malignant edema, of tuberculosis, and the bacillus pyocyaneus. The latter is extremely difficult to get rid of after it once enters a wound. The writer knows of a private room in a large hospital in which, during the winter months, almost every wound which suppurates becomes contaminated by this organism. Its surgical significance is that healing is slow.

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* By Chauncey P. Smith, M.D.

Schatz's Method. The hands and forearms are scrubbed with green soap (which is strongly alkaline), and a clean nail-brush, for from two to three minutes. The nails are cleaned. The hands and forearms are immersed in a warm, saturated solution of potassium permanganate, which has a somewhat germicidal effect, until stained a deep mahogany black, and are then decolorized with a warm solution of oxalic acid. It has been shown that both solutions are much more potent at a temperature of 40° C. The hands and arms are then washed in normal salt solution (.6 per cent.) and soaked in bichloride of mercury, 1–1000, four minutes, the solution being frequently changed, as the bichloride is decomposed by contact with the skin into an albuminate, which is worthless as a germicide. The decomposition is indicated by a milkiness which soon appears. The nail-brushes should be of the cheap variety—similar to the ordinary scrubbing-brush. They should be steamed for an hour after a day's work, and kept in a 3 per cent. carbolic acid solution when not in use. The objection to this plan is that it softens the brush.

Park's Method. The active germicide in this method is the essential oil of mustard. The hands and forearms are scrubbed with green soap and dry cornmeal, the latter taking the place of a nail-brush. They are washed in water. The nails are cleaned; the parts are then rubbed with a paste of mustard-flour and water until the mixture begins to sting. They are washed in water and rinsed in bichloride of mercury, 1–1000. The water must be cold during the manipulation of the mustard; the essential oil is only given off in cold water. The flour of mustard is employed, as it is a very convenient way of obtaining the oil. Only the best should be used, as the flour varies much in its efficiency.

Ligatures, Catgut.—The elaborate methods used for the preparation of catgut are due to fear of possible contamination of the gut with the spores of the bacillus of anthrax. The gut is prepared from the intestine of sheep (see Bacillus anthracis, Part IV.), and several well authenticated cases of anthrax infection from this source have been reported. Only the best German gut should be used, as the ordinary gut is covered with shellac, which interferes with penetration by chemicals.

Bissell's Method. Remove all labels and binders from the skein and soak it in a 1–1000 ethereal solution of bichloride of mercury for from four to six hours, depending on the size of the gut, the idea being that as the fat is absorbed by the ether the bichloride is carried into the gut. Wash with ether, to remove the excess of the bichloride, for three to five minutes; boil in 95 per cent. alcohol for fifteen minutes.

Note. If it is desirable to keep the gut on spools, it can be wound after the immersion in the bichloride. In making up solutions of bichloride of any strength for the preparation of gut do not use the ordinary tablets, as they are made up with ammonium chloride, which, on boiling, decomposes, and the products weaken the gut. Use only the pure crystals of corrosive sublimate.

Formaldehyde Catgut.—This gut possesses all the advantages of chromicized gut, including the length of time before absorption in the tissues, besides being stronger, less stiff and brittle. It can also be used in preference to kangaroo-tendon, the preparation of which is often imperfect. The writer knows of several cases where infection was caused by the kangaroo-tendon used. Formaldehyde catgut ties like silk; a knot once tied stays, and it does not swell on immersion in water.

Hoffmeister's Method. The gut is wound on spools, and soaked for twenty-four hours in a 4 per cent. solution of formaldehyde (Schering's formalin), and is shaken once in a while to get rid of small air-bubbles which adhere to the gut. Boil in water for 15 minutes and keep in 95 per cent. alcohol containing .1 per cent. bichloride of mercury and 5 per cent. of glycerin.

Silk.—Steam in sterilizer for one hour on the first day, and on the second and third day steam for one-half hour (Halsted).

Silkworm-gut.—As for silk or instruments.

Instruments.—Boil for five minutes in a 1 per cent. solution of sodium carbonate, as the salt itself is somewhat germicidal, and also, in this strength, prevents rusting of the instruments.

Dressings.—Pack loosely in a canvas bag, jar, or suitable vessel, and steam for one hour on the first day, one-half hour on the second and third days.

Sponges.—Beat well in a bag to remove the gross dirt, particularly the sand; boil a vessel full of a 1 per cent. solution of sodium carbonate, and remove from the flame; immerse the bagful of sponges in this solution, and allow it to become cool. This will take about one-half hour. Afterward, wash off the soda solution with sterilized water. Keep the sponges in 1–1000 solution of bichloride of mercury (Schimmelsbusch). The active agent in this sterilization is the boiling sodium carbonate solution. Bleaching weakens the sponge, and is of no practical value. The bichloride of mercury inhibits or prevents any possible contamination.

Skin of Patient.—Scrub with green soap, shave, and wash with equal parts of ether and alcohol to remove the fat. Then apply a green soap poultice for twelve hours. Before the operation scrub off the green soap, and wash down with equal parts of alcohol and ether. Scrub well with 1–1000 bichloride of mercury.

Park's method can also be used, as for the hands.

Drainage-tubes.—As for instruments, sponges or dressings.

Irrigating Solutions. Clean wounds.—The best, by far, is that which approximates the amount of salts in the blood-serum, namely, normal salt solution (.6 per cent.), and no other solution should be used on serous membranes. Sterilized water causes the endothelium to desquamate, while bichloride of mercury causes necrosis of the tissues, and hence both of them are to be avoided. If the wound be clean, there is no necessity for a germicide.

Infected Wounds.—Hydrogen peroxide is very efficient, as, in contact with pus, it is said to liberate oxygen, which in its nascent state is markedly germicidal. It also is said to have a peculiar affinity for leucocytes, and hence should not be used after granulation is well established (Morris).

Solutions of carbolic acid, 2 per cent., or bichloride of mercury, 1–1000, irritate the tissues greatly, and are, in the writer's opinion, of doubtful value. The bacteria in an infected wound are probably too deeply implanted in the tissues to be reached by solutions at all, and the antiseptic may be so diluted or chemically changed as to be valueless, to say nothing of the necrosis of the tissues produced. Bichloride of mercury changes into an albuminate. Stronger solutions not only destroy the bacteria, but also the cells of the tissues.

PART III.

NON-PATHOGENIC BACTERIA.

THE number of varieties of non-pathogenic bacteria is very large. Eisenberg, Bacteriological Diagnosis, edition of 1891, describes 376 species of bacteria, mostly non-pathogenic. Sternberg, Manual of Bacteriology, edition of 1893, enumerates 489 species, including the pathogenic varieties, but the majority, of course, are non-pathogenic. Probably some of the bacteria which have been described as distinct species are in reality not different; but, on the other hand, it is also probable that a still larger number of species have not been described at all; how many it is impossible to say. In a work of this character it is feasible to mention only a few of the commonest and best-known species of non-pathogenic bacteria.

Micrococcus agilis.—Found in water; coccus about 1 μ in diameter, usually appearing as diplococci, sometimes as streptococci and tetrads; liquefies gelatin slowly; grows slowly, only at room temperature, on ordinary culture-media, forming a rose-red pigment on agar and potato. This micrococcus is remarkable in being actively motile; it possesses a flagellum.

Micrococcus ureæ.—Found in decomposed, ammoniacal urine and in the air; coccus .8 to 1 μ in diameter, occurring singly or in various combinations; does not liquefy gelatin; aërobic and facultative anaërobic; grows rapidly, best at 30° to 33° C.; grows on ordinary gelatin, but best on special media; it decomposes urea, producing ammonia and carbon dioxide, which form ammonium carbonate.

Sarcinæ.—There is a large number of species of sarcinæ. They are common organisms in the air. They frequently contaminate plate-cultures. Many of the sarcinæ of the air present, in cultures, growths having brilliant colors, from which some of them are named; thus there are orange, yellow, rose-colored and white sarcinæ, and others.

Sarcina pulmonum.—Found in the sputum of a consumptive; 1 to 1.5 μ in diameter, occurring in tetrads or cubes of eight cells; aërobic; does not liquefy gelatin; grows slowly, best at ordinary temperatures, preferably upon gelatin. It decomposes urine with the formation of ammonia. It is said to form endogenous spores which are extremely resistant to heat.

Sarcina ventriculi.—Found in the stomachs of man and of animals; 2.5 μ in diameter, occurring in cubes of eight cells or more; it does not liquefy gelatin; aërobic; grows on ordinary culture-media; the growths tend to become yellow. Small numbers of sarcinæ may occur in the normal human stomach; the presence of large numbers indicates the existence of abnormal fermentative processes.

Bacillus fluorescens liquefaciens.—Found in water; appears as a small rod, actively motile; aërobic; liquefies gelatin; grows rapidly at ordinary temperatures upon the usual culture-media. It forms a pigment having a beautiful greenish-yellow fluorescence, best seen in transparent media; the growth on potato has a brown color.

Bacillus fluorescens putidus.—Found in water; a short rod with rounded ends; actively motile; does not liquefy gelatin; aërobic; does not form spores; grows rapidly at the ordinary temperatures upon the common media. Gelatin cultures give off a powerful, foul odor of trimethylamin. It produces a greenish, fluorescent pigment, best seen in transparent media; on potato the growths form a thin, gray to brown, slimy layer.

There are several other fluorescing bacilli, mostly found in water.

Bacillus Indicus.—Found by Koch in the stomach-contents of an ape in India; a fine short bacillus with rounded ends; aërobic and facultative anaërobic; liquefies gelatin; grows rapidly, best at 35° C. upon the ordinary media; produces a brick-red pigment. Very large doses injected into rabbits caused death in three to twenty-four hours.

Bacillus prodigiosus.—Widely disseminated in the atmosphere of certain places; a short bacillus with rounded ends, in form often nearly like the micrococci; aërobic and facultative anaërobic; not motile, as a rule; liquefies gelatin rapidly; grows rapidly, best at 25° C. on the ordinary culture-media; cultures on potatoes give off a foul odor of trimethylamin. A brilliant red color, which only develops in the presence of oxygen, appears in cultures. The pigment forms as granules outside of the bacteria.

Bacillus violaceus.—Found in water; a slim rod with rounded ends which may form threads; actively motile; aërobic and facultative anaërobic; liquefies gelatin rapidly; forms endogenous spores placed near the center of the bacillus; grows rapidly, and not at high temperatures, upon ordinary media, forming a deep, violet-colored pigment.

Bacillus amylobacter (Clostridium butyricum, Bacillus butyricus, Prazmowski).—Found widely distributed in nature in decomposing vegetable material and in the stomachs of ruminant animals; a large, thick rod with round ends, often arranged in chains; actively motile; anaërobic; forms spores, which are located in the center of the bacillus and give it a spindle-shaped form, or at one end when it has the outline of a tadpole; has not been cultivated satisfactorily on ordinary media; grows best at 35° to 40° C.; decomposes carbohydrates with the formation of butyric acid; decomposes cellulose.

Bacillus butyricus (Hueppe).—Found in milk; appears as a small, irregular rod, also forming threads; very actively motile; aërobic and facultative anaërobic; rapidly liquefies gelatin; forms centrally located spores; grows best at 35° to 40° C.; grows rapidly on ordinary media; coagulates milk, redissolving the coagulum, producing also butyric acid.

Bacillus megatherium.—Obtained by DeBary from cooked cabbage-leaves; a large bacillus with rounded ends, often forming chains; motile; slowly liquefies gelatin; aërobic; forms spores, especially in potato cultures; grows rapidly at room temperature on the ordinary media.

Bacillus mesentericus vulgatus (Potato bacillus).—Found on potatoes; a large, long rod with rounded ends, often forming long chains; motile; liquefies gelatin; aërobic; forms spores; grows rapidly, best at about 20° C.; grows on ordinary media, forming on potato a thin, wrinkled membrane which spreads rapidly over the surface. It coagulates milk. It possesses numerous flagella. The spores are extremely resistant to heat.

Bacillus phosphorescens Indicus.—Obtained from sea-water; a small, thick, rod-shaped bacillus with rounded ends, also forming threads; actively motile; liquefies gelatin; aërobic. It grows slowly, best between 20° and 30° C., upon the usual media. Its cultures, when old, especially when on animal nutrient-media and in the presence of certain sodium salts, are phosphorescent in the dark.

There are various other bacilli which produce phosphorescence, some of which do not liquefy gelatin.

Bacillus mycoides (Bacillus ramosus, *Wurzelbacillus*).—Found in the earth and in water; a large, short bacillus with rounded ends, often forming chains and threads; slightly motile; liquefies gelatin; aërobic; forms centrally located, oval spores; grows rapidly at room and incubator temperatures upon the usual media. It is said to rapidly decompose albumen with the formation of ammonia.

Bacillus subtilis (Hay bacillus).—Found on hay, in the air, water, ground and decomposing fluids; a large bacillus somewhat resembling the anthrax bacillus in form, but with rounded ends, often forming chains or long filaments; motile; possessing flagella; liquefies gelatin; aërobic. It may have large, centrally located spores, which form best on potato at about 30° C. The spores are extremely resistant to heat and to chemical germicides. It grows best at about 30° C. upon the ordinary culture-media. Bacillus subtilis may easily be isolated in pure culture by adding finely-cut hay to bouillon. Let it grow till spores form, then place portions in the steam sterilizer for five or ten minutes. Plates made from the bouillon will probably show colonies of the bacillus subtilis only, as the steam may be expected to have destroyed all organisms except its very resistant spores.

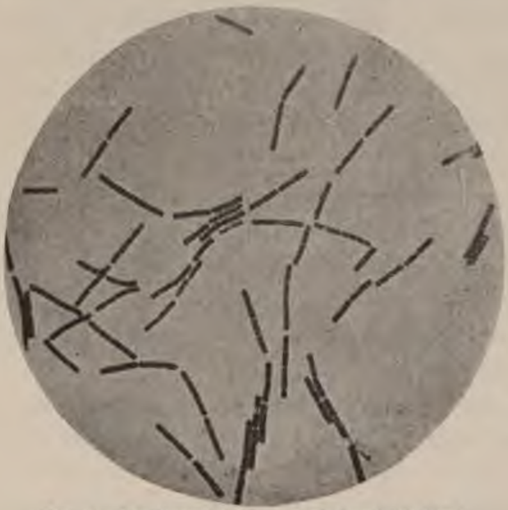

FIG. 46.—Bacillus subtilis. (Fränkel and Pfeiffer.)

Bacillus erythrosporus.—Found in decomposing fluids and water; a slim bacillus with rounded ends; motile; does not liquefy gelatin; facultative anaërobic; forms oval spores, two to eight in each filament; grows rapidly, only at ordinary temperatures; produces a greenish-yellow fluorescent pigment. On potato it forms a limited, reddish growth, becoming nut-brown.

Bacillus cyanogenus (Bacterium syncyanum, Bacillus lactis cyanogenus, Bacillus of blue milk).—A bacillus from 1 to 4 μ long; .3 to .5 μ broad; with rounded ends; motile; forms spores located at the ends; is aërobic; grows rapidly at ordinary and at incubator temperatures on the usual culture-media; does not liquefy gelatin; produces a grayish-blue pigment at ordinary temperatures; milk is not coagulated.

Bacillus acidi lactici (Hueppe).—Found in sour milk; a short, plump rod; not motile; does not liquefy gelatin; aërobic and facultative anaërobic; forms spores located at the ends; grows slowly on the ordinary media; in milk causes development of lactic acid with precipitation of casein and production of gas.

There are numerous other bacteria, such as the bacterium acidi lactici, which cause the formation of lactic acid in milk.

Bacterium ureæ.—A short, thick bacillus with rounded ends; aërobic and facultative anaërobic; found in ammoniacal urine; grows slowly at room temperature upon gelatin, which is not liquefied; decomposes urea, forms ammonium carbonate.

Bacterium Zopfii.—Found in the intestines of hens; a bacillus .75 to 1 μ broad and 2 to 5 μ long; may form threads. Actively motile; does not liquefy gelatin; aërobic; forms spores; grows rapidly, best at 20° C. upon gelatin; it is said not to grow on blood-serum.

Spirillum rubrum.—Found by Esmarch in the putrefying cadaver of a mouse; short spirals twice the breadth of the cholera spirillum, usually with one to three turns; in bouillon growing into long spirals; motile with flagella; spore formation probable; does not liquefy gelatin; grows slowly, best at about 37° C. on the ordinary media; produces a wine-red pigment only when the air is excluded.

Spirillum or Spirochæta dentium.—Found in the mouths of healthy persons, on the margins of the gums when they are covered with a dirty deposit; long spirals with several windings uneven in thickness; has not been cultivated.

Spirillum sputigenum.—Found in the human mouth in healthy persons at the margin of the gums; curved rods or short spirals which resemble the spirillum of cholera in form; has not been cultivated.

Spirillum rugula (Vibrio rugula).—Found in swamp water, in fecal matter, and in the tartar of the teeth; a curved rod .5 to 2.5 μ broad and 6 to 8 μ long, having one flat spiral winding; motile, with flagella at the ends; liquefies gelatin; anaërobic; forms spores located at the ends; develops rapidly, best above 20° C., on most media, making a whitish growth; gelatin colonies yellowish-white; developing a fecal odor in all cultures.

Spirillum volutans.—Found in swamp water; very long spirals with several turns 1.5 to 2 μ broad and 25 to 30 μ long; motile, with a flagellum at each extremity. The protoplasm is granular.

Spirillum undula.—Found in putrefying infusions containing organic matter; a rather short spiral form with three turns or less, about 1 μ thick and 8 to 12 μ long; actively motile, with a flagellum at each extremity.

Spirillum or Spirochæta plicatile.—Found in swamp water; spiral forms of various lengths; sometimes 100 to to 200 μ long; actively motile.

The spirilla (vibrios or comma-shaped forms), closely resembling the spirillum of cholera, will be considered in connection with that organism.

Higher bacteria.—Certain organisms (beggiatoa, thiothrix, leptothrix, cladothrix, streptothrix) of more complicated structure than most bacteria, but resembling them in many respects, are called " higher bacteria." They consist of definite filaments which are usually made up of rod-shaped elements, but the relation between these elements is very intimate. Some of them (beggiatoa, thiothrix) contain sulphur granules. Many of them occur in water. There are forms among them which are found attached to some object by one end of the filament (thiothrix). Some of them (streptothrix) have branching filaments, which are very rarely seen among the lower bacteria (see page 89). Often one end of the filament becomes specialized for the purposes of reproduction. The fungus of actinomycosis probably belongs to the higher bacteria.

Leptothrix buccalis.—Found in the mouth cavity. This name has been applied to large, twisted, thread-like organisms, in which segments can be demonstrated with difficulty or not at all. Apparently different organisms have been described under this name. Vignal claims to have cultivated a leptothrix buccalis. Miller recognizes two principal species, neither of which could be cultivated,—*leptothrix innominata*, which shows no transverse divisions, and which is stained faintly yellow by iodine; and *bacillus buccalis maximus*, in which the transverse divisions are distinct, and which is stained brownish-violet by iodine. Miller's *leptothrix maxima buccalis* is similar to the last except in lacking the iodine reaction.

In the course of bacteriological work one constantly encounters yeasts and moulds, which, although not bacteria,

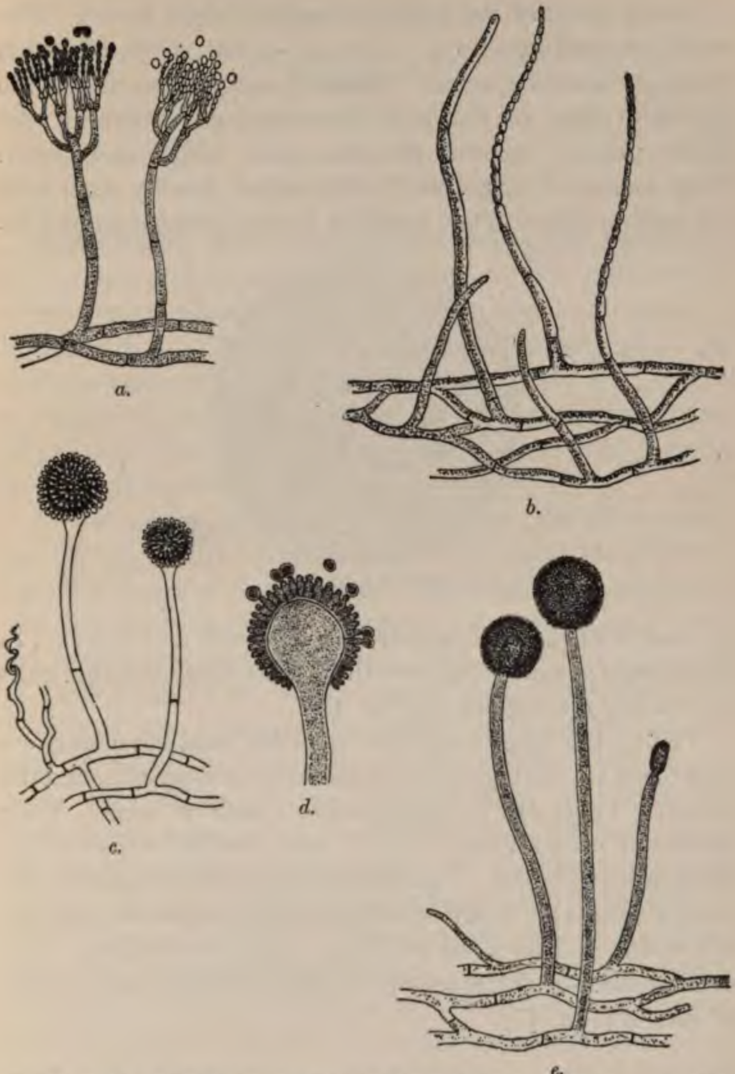

FIG. 47.—*a.* Penicillium glaucum. *b.* Oïdium lactis. *c.* Aspergillus glaucus. *d.* The same more highly magnified. *e.* Mucor mucedo (Baumgarten).

must nevertheless be understood and recognized to avoid error. Accidental contamination of tubes or plates is likely to be the result of the growth of some of these forms. The yeasts generally go by the name of *saccharomyces*, of which there are several species. The *saccharomyces cerevisiæ* is the ordinary yeast of alcoholic fermentation. Some of the yeasts present colored growths—red, white and black. They consist of large, oval cells, which readily stain with the aniline dyes. They multiply by the protrusion of a little bud from the cell, which develops into a new cell. In an actively germinating growth of yeast these budding cells are readily distinguished (Fig. 48).

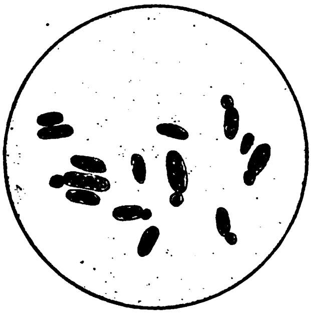

FIG. 48.—Yeast cells.

Among the other fungi, the varieties most commonly encountered are the *mucor*, the *penicillium*, the *aspergillus* and the *oïdium*. There are various species of each of them. They consist of cells arranged end to end, making a thread-like body called a *hypha*. The threads are matted together and form a *mycelium*. Certain threads project upward from the mycelium, and on them are borne *spores*, or conidia. The arrangement of the spores is characteristic in each variety of mould (Fig. 47).

PART IV.

PATHOGENIC BACTERIA.

Suppuration and Allied Conditions.—The occurrence of suppuration is characterized by certain appearances which we are accustomed to describe under the name of inflammation. The study of inflammation belongs to pathology, and cannot be considered here. However, certain evidences which are characteristic of the suppurative variety of inflammation need to be studied on account of their relation to the action of the pyogenic bacteria.

In a suppurating area, as is well known, the blood-vessels are dilated, and the lymph-spaces become filled with serum. Leucocytes are attracted to the neighborhood in large numbers, we may suppose by a positive chemotaxis, and crowd the small veins and capillaries. The leucocytes, by reason of their ameboid movement, pass through the walls of the vessels at little openings filled with cement-substance, situated between the lining endothelial cells. According to the theory of phagocytosis, they are bent on finding the irritant which has led to the inflammation, and upon isolating it and rendering it harmless. At the point which appears to be the center of the inflammatory area there is usually, but not always, a necrosis of the cells of the tissue; this constitutes the central slough or the familiar core of some boils. The necrosis is to be attributed to poisons formed by the micrococci. In sections cut through such an abscess the nuclei of the central necrotic cells fail to take the nuclear stain; the necrotic mass does not stain, or takes the dye diffusely and irregularly, and it exhibits many fine granules.

We find the cells of the tissues surrounding the necrotic area mingled with large numbers of polynuclear leucocytes, which enclose the area of irritation, as an army of defence might be drawn up to repel an invasion.

The nuclei of the cells near the center of the abscess are frequently broken up into a number of small parts (fragmentation), which indicates the commencement of their destruction. In sections through small abscesses it is possible, by means of a double stain of borax-carmine, followed with gentian-violet, according to Gram's method, to bring out the histological character of the tissue, and at the same time to stain the common pyogenic bacteria, which are usually found near the center of the abscess in large numbers, even making masses visible with a low power of the microscope. Preparations, most convincing and of great beauty, may be secured in this manner. It is often possible to demonstrate masses of micrococci filling up the lumina of capillaries in which they are lodged as emboli.

The production of pus in the center of the abscess is due to the liquefaction of the necrotic tissue, which apparently results from the action of some peptonizing ferment. In the liquid thus formed, immense numbers of the polynuclear leucocytes are found floating, and they constitute the vast majority of the so-called *pus-cells.* The nuclei of these cells are obscured by clouds of extremely fine granules. The granules are of an albuminoid nature, and are dissolved by acetic acid, when the nuclei become visible. The nuclei are generally in three, four, five or more parts. The presence of the fine albuminoid granules in the pus-cells is to be counted as a degenerative change. Although it is possible to produce suppuration in laboratory experiments by the introduction of sterilized irritants, such as croton oil, in the vast majority of cases suppuration is due to the action of pyogenic bacteria.

Specimens of pus will nearly always be found to contain bacteria, which can be demonstrated by cultivation, and, as a rule, also in smears made and stained upon cover-glasses. The bacteria are generally found outside the pus-cells. In the case of the gonococcus and the diplococcus meningitidis intracellularis they are characteristically found in pairs, inside of, or at least attached to the pus-cells. The character of the suppuration differs somewhat with the different species of pyogenic bacteria. The kind of abscess above described —localized and having a central slough, usually rather slow in progress—is typical for the staphylococcus pyogenes aureus, which is prone to produce circumscribed areas of suppuration. The streptococcus pyogenes, on the other hand, oftener leads to suppuration of a more diffused character, such as we see in cellulitis and erysipelas; but either organism may, at times, produce the effects usually characteristic of the other. Pus having a blue or green tinge generally owes the color to the presence of the bacillus pyocyaneus. The commonest pus-producing organism is then the *staphylococcus pyogenes aureus*, and next to that the

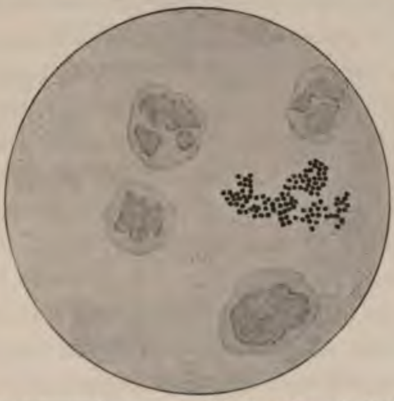

FIG. 49.—Pus from an acute abscess stained by Gram's method, showing Pus-cells and Staphylococcus pyogenes aureus.

streptococcus pyogenes. Among the other pyogenic bacteria teria the following may be named:

Staphylococcus pyogenes albus, including staphylococcus epidermidis albus; streptococcus of erysipelas (probably identical with streptococcus pyogenes); gonococcus; diplococcus intracellularis meningitidis; staphylococcus pyogenes citreus; micrococcus tetragenus; micrococcus pyogenes tenuis, which may be the same as the micrococcus lanceolatus; staphylococcus cereus albus and flavus.

Pus-formation may also be due to micrococcus lanceolatus, bacillus pyocyaneus, bacillus proteus, bacillus coli communis, bacillus pyogenes fetidus, bacillus pneumoniæ (of Friedländer), the ray fungus of actinomycosis, and possibly the bacillus of bubonic plague. Besides these organisms, there are others whose effects are usually more marked in a specific way which sometimes form pus, as the bacilli of diphtheria, tuberculosis, glanders and typhoid fever.

Frequently two or more species of pyogenic bacteria will be found associated.

The table on page 185, quoted from Dowd, shows the frequency of the occurrence of various pyogenic bacteria in 135 cases of different types of suppuration.

The condition of the animal's tissues is of great importance in determining whether or not suppuration is to occur. It will be seen that we are repeatedly subjected to infection with pyogenic bacteria, but that in most cases suppuration nevertheless does not occur. The local conditions have an important influence in determining infection. Regions of hyperemia, edema, anemia or necrosis are especially liable to suppuration, as are tissues which have been bruised, lacerated, strangulated or otherwise damaged. Furthermore, the general condition of the patient is of great importance. Chronic diseases and conditions of exhaustion or depression dispose to suppuration, and the depraved condi-

	Cellulitis, 51 Cases.	Infected Fresh Wounds, 17 Cases.	Old Granulating Wounds, 18 Cases.	Healing Wounds: Stitches, 5 Cases.	Furuncles, 7 Cases.	Abscesses, 37 Cases.
Streptococcus pyogenes alone	9	3				8
Streptococcus pyogenes predominant	23	3				8
Streptococcus pyogenes relatively few	3	1	6			1
Staphylococcus pyogenes aureus alone	11	1	1	1	7	6
Staphylococcus pyogenes aureus predominant	8	2				1
Staphylococcus pyogenes aureus relatively few.	13	3				2
Staphylococcus pyogenes or epidermidis albus alone	1	4	2	4		2
Staphylococcus pyogenes or epidermidis albus predominant		1				
Staphylococcus pyogenes or epidermidis albus relatively few	10	5	3			6
Staphylococcus cereus albus	3	1	2			1
Staphylococcus citreus	1		2			1
No growths on agar						11
Very few growths on agar			3			3
Bacillus pyocyaneus			1			3
Bacillus coli communis						3
Overgrown	4		2			1
Few undetermined colonies	12	2	5			5

tion of the tissues in diabetes renders the sufferer from this disease especially liable to it. These facts have already been enumerated in a previous chapter (page 132). In the lower animals we find that it is often very difficult to produce suppuration artificially with the ordinary pyogenic bacteria. In rabbits the subcutaneous introduction of staphylococcus pyogenes aureus frequently fails to produce an abscess. Suppuration is likely to result, however, if an irritant body like a piece of sterilized potato or sterilized glass be introduced along with the bacteria.

Pyogenic bacteria are most frequently introduced into the body through the agency of injuries and wounds of various sorts. They are very widely disseminated in nature, and are always liable to be clinging to external objects, especially in cities and around dwellings. The infection of a wound in this manner, when the suppuration is of a spreading character, such as is most characteristic of streptococcus infection, is known in every-day language as "blood-poisoning." It is possible for infection to take place around hair-follicles through the unbroken skin. In such instances the suppurative inflammation first shows itself in a minute red pimple with a hair in the center. The pimple presently becomes a pustule. The process may cease at this point, or it may be only the commencement of a large carbuncle with a central slough. Such infection has been produced experimentally on the human skin by rubbing in cultures of staphylococcus pyogenes aureus. It is, furthermore, the constant experience of post-mortem examiners that infection may occur around the hair-follicles when no wound of the skin has been inflicted.

In many instances, infection with the pyogenic bacteria follows upon some preëxisting infection; this happens, for instance, in tuberculosis, when tuberculous lungs become infected with streptococcus pyogenes, leading to the formation of a cavity. It is a common occurrence in gonorrhea, after the acute stage of the disease has passed, when we find the gonococcus in the pus, mingled with other pyogenic micrococci. Secondary infection with pyogenic bacteria is usually due to the streptococcus pyogenes, often also to the micrococcus lanceolatus. Sometimes we are obliged to admit that the manner in which the pyogenic bacteria enter the body is unknown.

The severe general symptoms, familiar to every physician, often accompanying acute suppuration, indicate the formation of toxic bacterial products and their absorption. Experimental evidence of the formation of such toxic products is not so clear, however, for the pyogenic organisms as for some of the other bacteria. It has been shown that the filtrates of cultures of staphylococcus pyogenes aureus, freed from the bacteria, are capable of producing suppuration in the lower animals.

The pyogenic bacteria play a somewhat different part in producing disease, which is fully as important as the typical suppuration seen in an abscess. This happens when the suppurative condition is mixed with other phenomena, or when there is inflammation of another variety without suppuration at all; or there may be lesions not inflammatory in a strict sense. These differences in their action depend largely upon the organ affected. One such condition is osteomyelitis, which is, usually, suppuration occurring in bone, but which does not present the ordinary picture of pus-formation owing to the hard and unyielding character of the tissue. Other conditions of very great importance are meningitis, pericarditis, pleuritis, pneumonia (croupous and broncho-), peritonitis and endocarditis. It will be observed that these affections are, for the most part, inflammations of the serous membranes. Such inflammations, when they are produced by pyogenic bacteria, are likely to be of great severity, accompanied by the formation of fibrinous exudates; pus-formation may or may not be present. We find that the cause at times is the staphylococcus pyogenes aureus; this is often the case in malignant endocarditis. Generally speaking, however, in such inflammations the streptococcus pyogenes and the micrococcus lanceolatus occur most commonly, although they are by no means the only organisms found.

From a point where there is suppuration or other localized infection, pyogenic bacteria may enter the circulation and become widely disseminated throughout the body. That happens very commonly in malignant endocarditis. In this manner secondary or metastatic abscesses may be produced in the most diverse organs.

The term *pyemia* is used to describe the dissemination of pyogenic bacteria in the circulating blood, with the formation of metastatic abscesses.

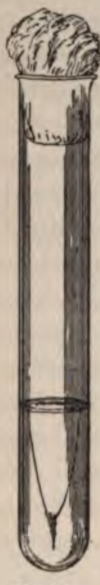

FIG. 50. Staphylococcus pyogenes aureus, culture in gelatin showing liquefaction.

Staphylococcus pyogenes aureus.—A micrococcus of variable size, arranged in irregular clumps, sometimes in pairs; about .8 to .9 μ in diameter; not motile (Fig. 49). It stains by Gram's method; it is facultative anaërobic; grows rapidly, best at 30° to 37° C. It liquefies gelatin. A soluble peptonizing ferment has been separated from cultures which acts by itself, independently of the living micrococci. Upon gelatin plates small colonies appear at the end of about two days. It grows well upon all the culture-media. Milk is coagulated. It does not lead to fermentation with the production of gas.

The growths in the first place are pale, subsequently becoming golden-yellow in color. This color appears well on all media, and is especially distinct on potato. Sometimes the color is slow in developing.

In a fresh, moist condition the organism is killed by ten minutes' exposure to 58° C.; in a desiccated condition it requires a temperature of 90° to 100° C. to destroy it. It resists drying in a considerable degree. In the same specimen the micrococci may have quite different resisting-powers to chemical germicides. Some of them are destroyed by 1–1000 solution of bichloride of mercury in five minutes; others survive exposure to the same for from ten to thirty minutes. (Abbott.)

As has already been mentioned, the staphylococcus pyogenes aureus is the commonest of the pyogenic bacteria in man. It has been obtained from a great variety of sources, and appears to be able to exist as a saprophyte. It has been found on the skin, in the mouth, in the nasal and pharyngeal mucus, and also in the alimentary canal. It has furthermore been detected in the air and in dust. It appears to find the conditions necessary for its existence in the vicinity of human habitations.

Cultures of the staphylococcus pyogenes aureus vary considerably in virulence. These variations are sometimes to be explained through cultivation on unfavorable media or repeated transplantation from one medium to another; but at times the diminished virulence is due to unknown causes. The lower animals used for experiments are not as readily infected as man. The local introduction in rabbits or guinea-pigs of a part of a culture of staphylococcus pyogenes aureus may be entirely without effect. The use of a very large dose, or the addition at the same time of some kind of irritant, may produce an abscess. Large amounts of cultures in bouillon may often be injected into the peritoneal cavity of the dog without effect, when the simultaneous addition of a piece of sterile potato or an injury to the gut may lead to fatal peritonitis. Introduction of fluid-cultures into the venous circulation of the rabbit generally produces metastatic abscesses in the kidneys, the heart-muscle and the voluntary muscles, and causes death.

In man this organism produces suppuration of a localized character, such as we are familiar with in boils and carbuncles. It has been shown to be the usual cause of infectious osteomyelitis. Osteomyelitis has been produced experimentally in rabbits by the injection of the staphylococcus pyogenes aureus, both with and without previous injury to the bone of the animal. Ulcerative endocarditis has on numerous occasions been shown to be due to this organism. It has been found possible to produce ulcerative endocarditis experimentally in animals by the injection of the staphylococcus pyogenes aureus when the valves of the heart have first been mechanically injured. The staphylococcus pyogenes aureus has also been found in acute abscesses of the lymph-nodes, tonsils, parotid gland, and mammary gland, in suppurating joint affections and empyema. It appears, furthermore, in acute inflammation of the serous membranes,—pleuritis, pericarditis, peritonitis,—although less frequently than the streptococcus pyogenes.

Staphylococcus pyogenes albus.—In form and manner of growth this organism behaves like the staphylococcus pyogenes aureus, with the exception that it produces no colored growths and its cultures appear white. Its pathogenic properties are less marked, and it is a less frequent cause of suppuration than the staphylococcus pyogenes aureus. It has, however, been found in acute abscesses on numerous occasions.

Staphylococcus epidermidis albus. — According to Welch, the epidermis of man contains with great regularity the organism to which he gave the above name, and which he considers to be a variety of staphylococcus pyogenes albus. It grows, liquifies gelatin, and coagulates milk more slowly than the ordinary staphylococcus pyogenes albus. It is, furthermore, possessed of less marked pus-producing tendencies. Welch found it impossible to sterilize the skin so as to remove this micrococcus from it. The organism is usually innocuous. It has been found in healthy wounds on numerous occasions. It is capable of causing trouble in wounds when necrotic or strangulated tissues are present, or where a foreign body like a drainage-tube has been left in the wound. It is a common cause of stitch abscesses.

Streptococcus pyogenes.—Appears as micrococci arranged in chains, often in pairs, when the adjacent cocci may be flattened. Sometimes the chains are very long. The diameters of the cocci vary from .4 to 1 μ. Attempts have been made to create varieties of streptococci according to the length of the chains. On that basis a streptococcus brevis and a streptococcus longus have been described.

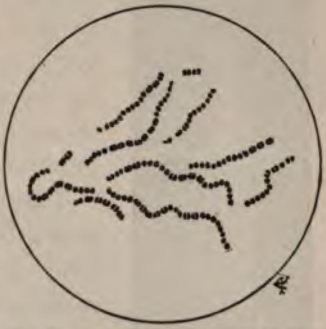

FIG. 51.—Streptococcus pyogenes, from a pure culture.

The streptococcus pyogenes is not motile. It stains by Gram's method. It is facultative anaërobic; grows best in the incubator; more slowly at room temperature, and does not liquefy gelatin. In gelatin plates it produces small, round, white, punctiform colonies which are slow of development, and are only visible after about three days. It grows on the ordinary media; with the exception of potato, according to some authors. Milk may or may not be coagulated. The growths are never very luxuriant, and may die out entirely after a few transplantations.

It is killed by exposure to 52° to 54° C., in ten minutes. The streptococcus pyogenes occurs frequently on the mucous surfaces of the healthy body. It is often found in pus, especially pus of spreading inflammations of the kind known as cellulitis. This organism is the usual infectious agent in puerperal fever, metritis and peritonitis. It has been discovered many times in ulcerative endocarditis. It is frequently present in the false membrane found in genuine diphtheria. It is also the cause of many of the pseudomembranous or so-called "diphtheritic" affections of the throat where the Klebs-Löffler bacillus of diphtheria is wanting. These cases may be indistinguishable clinically from genuine diphtheria, and their nature will only be revealed on bacteriological examination. They are, however, as a rule, milder than the genuine diphtheria. The pseudo-membranous affections of the throat which occur in scarlet fever and measles are generally caused by the streptococcus pyogenes, although those diseases may be complicated by genuine diphtheria.

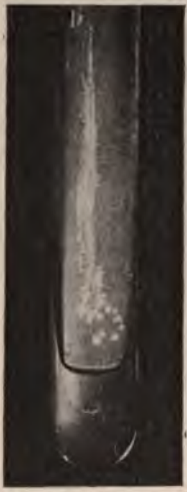

FIG. 52.—Streptococcus pyogenes, culture on agar. (Fränkel and Pfeiffer.)

The streptococcus pyogenes is pathogenic for mice and rabbits, but the virulence is very variable. Its virulence is rapidly lost in artificial cultures. It is said that it is best maintained when cultures on gelatin, after forty-eight hours' growth, are kept in a cool place, as in the ice-chest. Marmorek undertakes to maintain or increase the virulence by growing it first in a mixture of human blood-serum (or that of the ass or the horse) with bouillon, and then inoculating it into the body of a rabbit, alternating these procedures to obtain a culture of very high virulence. A serum of uncertain value, derived from the horse and intended to cure streptococcus infection, has been prepared by Marmorek.

Coley has recommended a bouillon culture of streptococcus pyogenes (or of erysipelas), in which the bacillus prodigiosus was afterward grown, to be administered by injection, after sterilization of the cultures by heat, in cases of inoperable sarcomatous tumors. These injections appear in some cases to have accomplished remarkable and wholly unexplainable cures.

Streptococcus of erysipelas.—A streptococcus has been derived from cases of erysipelas which in all essential respects, in its morphology, its growth on culture-media, its behavior with stains, and its pathogenic properties, is similar to the streptococcus pyogenes. It is probable that these organisms are identical.

Micrococcus tetragenus.—Found in the cavities in the lungs of pulmonary tuberculosis, in sputum and in pus. The micrococci are enclosed in a transparent capsule, best seen in preparations from the tissues of inoculated animals, and are arranged in pairs or in fours; about 1 μ in diameter; not motile; stain by Gram's method. It grows well at the room temperature, but rather slowly; is facultative anaërobic; does not liquefy gelatin. Gelatin-plates show little, white, punctiform colonies, which, with the low power, are finely granular, and have a peculiar glassy shimmer; and in stab-cultures the growths appear as little colonies along the line of puncture. On agar, round white colonies form, not spreading. It produces a thick, slimy film on potato and a broad, white, moist growth on blood-serum. This

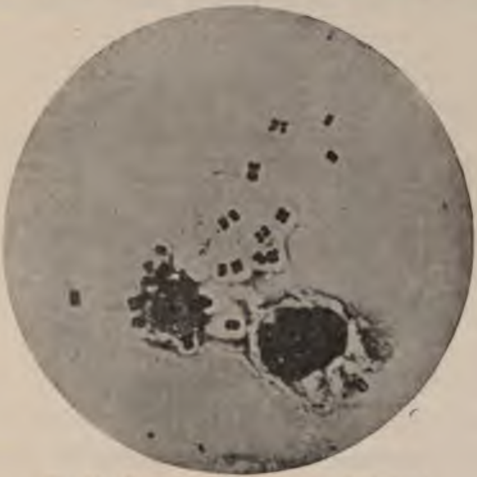

FIG. 53.—Micrococcus tetragenus. (Günther.)

organism is only occasionally found in pus. It is pathogenic to white mice and guinea-pigs, not to gray mice and rabbits. It may produce a septicemia or only a localized suppuration in guinea-pigs. In white mice a general septicemia results, when the micrococcus tetragenus is found in the blood and in the great viscera. White mice usually die in from two to six days; guinea-pigs in from four to eight days.

Micrococcus lanceolatus (Micrococcus pneumoniæ crouposæ, Micrococcus Pasteuri, Diplococcus pneumoniæ, Micrococcus of sputum septicemia, Streptococcus lanceolatus Pasteuri, and Pneumococcus of Fränkel).—This organism was discovered by Sternberg in his saliva in 1880, and afterward demonstrated to be the cause of lobar pneumonia by Fränkel and Weichselbaum. The micrococci usually occur in pairs. The pair of micrococci, in its most typical form, appears like a couple of curved triangles with their bases close to each other. The outline is usually described as being lancet-shaped. The micrococci are frequently oval or round; they often form chains. When it is most charac-

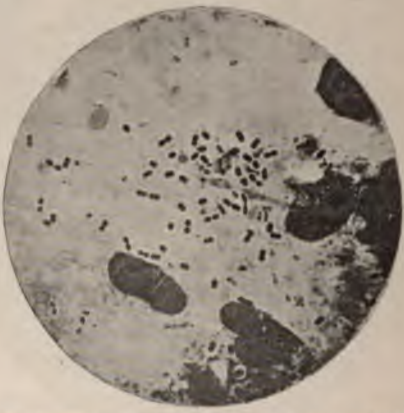

FIG. 54.—Pneumococcus of Fränkel. (Fränkel and Pfeiffer.)

teristic, each pair of micrococci is surrounded with a capsule, which is best shown in preparations made from the blood of infected animals or from pneumonic sputum; the capsule is not usually seen in preparations made from cultures. The pneumococcus is not motile. It stains by Gram's method, which also is useful in demonstrating the capsule. It is aërobic and facultative anaërobic. It grows only at elevated temperatures, preferably about 35° to 37° C. Gelatin is not liquefied. It grows well upon agar and upon blood-serum. It does not grow upon potato. Milk usually becomes acid, and is coagulated by it. The colonies are seen in their characteristic form upon agar, and are developed after about forty-eight hours, appearing as minute, whitish, translucent, circular growths.

It is killed by an exposure to 52° C. for ten minutes.

It is best cultivated from the blood of an animal which has been infected with the sputum of a case of lobar pneumonia. Cultures need to be transplanted every few days; they cannot usually be propagated more than a couple of months.

The virulence of the organism for animals diminishes rapidly in cultures. In cultures it frequently grows as a streptococcus. When virulent, it is pathogenic to mice and rabbits, less so to guinea-pigs. In these animals it is likely to lead to inflammations, and to rapidly fatal septicemia (twenty-four to forty-eight hours). The blood may contain great numbers of the diplococci. It may be introduced subcutaneously or into the peritoneum, or by intravenous injection when liquid cultures are used. Its virulence is very variable. In the sputum of a case of lobar pneumonia, early in the disease, it is likely to be virulent. The virulence is best maintained by repeated inoculations into mice or rabbits.

This organism is detected very frequently in the human mouth. When taken from the mouth it is not, however, pathogenic to animals in many instances, being found virulent in only from 15 to 20 per cent. of human mouths. It is the specific cause of croupous or lobar pneumonia in man. In that disease the characteristic lesion consists of an inflammation of the lung, involving large areas, usually one or several lobes. An exudate is poured into the air-vesicles, which in the early part of the disease contains red blood-cells, imparting the rusty color to the sputum. The principal element in the exudate is fibrin. The formation of fibrin produces the liver-like consolidation or "hepatization." The diplococci can readily be demonstrated in sections of pneumonic lung, which are best stained by carmine and gentian-violet, by the Gram-Weigert method. Although the exudate at first contains many red blood-cells and the solid lung appears red, subsequently it becomes decolorized and presents a gray color. Many leucocytes will now be found to have migrated into the air-vesicles, and the lung will have become relatively anemic, instead of hyperemic. Finally, the fibrinous exudate and the cells entangled in it become softened and liquefied. Some of this liquefied exudate is absorbed into the lymphatics in the walls of the air-vesicles; part of it is expectorated.

The micrococcus lanceolatus can be detected in large numbers, sometimes almost unmixed with other bacteria, in the rusty sputum of lobar pneumonia, often showing the peculiar unstained capsule. On account of its liability to be mixed with other forms of bacteria, its presence in the sputum of cases suspected of being pneumonia is not of very great value in differential diagnosis, especially considering that it is so commonly present in the normal mouth.

The micrococcus lanceolatus is often also the cause of broncho-pneumonia and of meningitis. It produces inflammations in other situations as well, the most important being pleuritis, pericarditis and endocarditis. The micrococcus lanceolatus may produce suppuration, although not very commonly.

G. and F. Klemperer claim to have obtained toxins from cultures of the pneumococcus, and to have established immunity in animals with the development in the blood of antitoxic substances. Their experiments have been repeated by Washbourn and others, but the interpretation of them at the present time is not clear.

The organism described by Rosenbach, under the name of the *micrococcus pyogenes tenuis*, is probably only a variety of the micrococcus lanceolatus.

Diplococcus intracellularis meningitidis.*—Found in the exudate of cerebro-spinal meningitis by Weichselbaum; a micrococcus about the size of the common pyogenic cocci; grows in pairs or fours, more often in pairs consisting of two hemispheres separated by an interval which does not stain; usually found within the pus-cells, in which respect it resembles the gonococcus. It is stained by ordinary methods with the aniline dyes, and is decolorized by Gram's method. It does not grow at the room temperature but only in the incubator; gelatin is not available. There is no growth on potato and

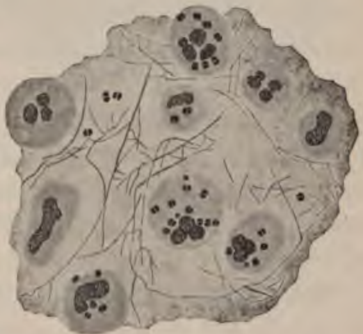

FIG. 55.—Diplococcus intracellularis meningitidis and pus-cells. (Councilman.)

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* The writer is indebted for the brief statement, which it is possible to give here, chiefly to the recent exhaustive report to the Massachusetts Board of Health by Councilman, Mallory and Wright.

scanty growth on agar or in bouillon. The development is most abundant upon Löffler's blood-serum, when round, white, shining, viscid-looking colonies with sharp outlines may be seen in twenty-four hours. The serum is not liquefied. Upon agar, or, better, upon glycerin-agar, the colonies are flat, round, translucent, viscid-looking, under the low power having a yellowish-brown color. The organism should be transplanted to fresh media frequently, as it rapidly loses its power of reproduction. Many of the tubes inoculated with the original material or with pure cultures show no growth.

It is moderately pathogenic for guinea-pigs and rabbits when inoculated into the pleura or peritoneum. Meningitis and encephalitis have been produced in the dog and goat by inoculation in the meninges.

This organism appears to be the principal if not the only cause of epidemic cerebro-spinal meningitis. The lesion consists of a purulent inflammation of the pia and arachnoid, extending into the brain substance, over the cord, and along the nerves. General invasion of the tissues of the body seems not to occur, but focal areas of pneumonia may be present. Spinal puncture in the lumbar region is recommended as a means of diagnosis. The puncture should be made early, and the fluid should be examined with the microscope and by cultures.

Micrococcus gonorrheæ (Gonococcus of Neisser).—Found in pus in cases of gonorrhea. The micrococci generally are in pairs, occasionally in groups of four. The cocci are flattened, the flattened sides face each other, and they are often compared to a pair of biscuits. The long diameter of the pair of biscuit-shaped elements is about 1.25 μ. The organisms are usually found attached to the epithelial cells or inside of the pus-cells; they are also found in smaller numbers floating free in the fluid. They stain with ordinary aniline dyes, for example Löffler's methylene-blue, but not by Gram's method.

The occurrence, (1) inside of the pus-cells, (2) of pairs of biscuit-shaped micrococci (3) which are not stained by Gram's method, will serve to distinguish the gonococcus from all the other ordinary pus-forming bacteria. There are other diplococci (pseudo-gonococci), probably non-pathogenic, which have been found in the urethra, which, it is said, are also decolorized by Gram's method. Such organisms are not likely to present all the points mentioned as characteristic of the gonococcus. The recognition of the gonococcus in the discharges of a case of acute gonorrhea is usually a matter of the greatest possible ease. It must be admitted, however, that in cases having chronic discharges, when its detection is most to be desired, the diagnosis may become very difficult and is frequently impossible, owing to secondary infection with the ordinary pus-forming or other bacteria, which may be present in larger numbers than the gonococcus itself.

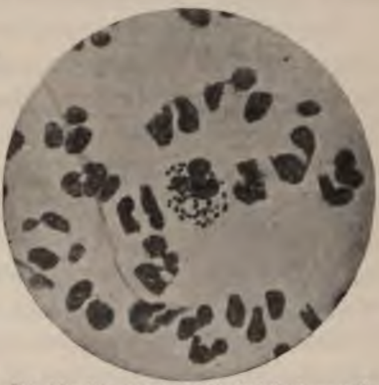


FIG. 56.—Gonococcus and nuclei of pus-cells. (Photo-micrograph by Dr. F. C. Busch, Pathological Laboratory, University of Buffalo.)

The gonococcus is a facultative anaërobe. It grows only in the incubator, and cannot therefore be cultivated upon gelatin. Its cultivation is in fact a matter of some difficulty. The medium usually selected is a mixture of agar with human blood-serum. The blood-serum from the placental blood and from pleuritic and peritoneal transudates and hydrocele fluid, has been taken. The addition of human urine, sterilized by filtration through porcelain, to the mixture of blood-serum and agar improves its character according to some writers. A convenient medium is one consisting of one part of human serum derived from a pleuritic effusion, added to two parts of a 2 per cent. nutrient agar. The agar has previously been sterilized; the two are mixed in tubes while fluid; they are cooled while in an inclined position, and are sterilized between 65° and 70° C. by the fractional method on six consecutive days. They are afterward tested in the incubator for two days.

The colonies of the gonococcus are very small, grayish-white, circular, translucent; appearing after from twenty-four to forty-eight hours. They may attain a diameter of 1 to 2 m.m. The gonococcus will occasionally develop on ordinary glycerin-agar or Löffler's blood-serum medium, but the growth is likely to be feeble and cannot be relied on. The cultures live for a considerable time if kept from drying. The gonococcus is not known to produce urethritis or conjunctivitis in any of the lower animals. In the peritoneum it may cause suppurative inflammation in mice and guinea-pigs. Reproduction of the disease in man has been effected by experimental inoculation with pure cultures. Besides being the cause of gonorrheal urethritis, the gonococcus has been isolated from cases of vaginitis in women and little girls, and from gonorrheal conjunctivitis. It has been found to be the cause of many cases of pyosalpinx, as well as of gonorrheal proctitis, arthritis, myocarditis and endocarditis; these conditions complicating gonorrhea may also be secondary or mixed infections.

Bacillus pneumoniæ (of Friedländer).—A short bacillus with rounded ends, sometimes growing out to a greater length; sometimes occurring in pairs; surrounded by a capsule which is only seen in preparations made from the tissues of infected animals, and is not demonstrated in cultures. This bacillus is not motile. It does not form spores. It stains with the ordinary aniline dyes, but does not stain by Gram's method. It is aërobic and facultative anaërobic. It may be cultivated at ordinary temperatures, but grows better in the incubator. It does not liquefy gelatin. Stick-cultures in gelatin develop especially at the point where the puncture enters the surface of the gelatin, making what is called a "nail-shaped" growth; the growth in gelatin is white. It develops also on the other media. Dextrose and lactose are fermented by it; in cultures on potato, gas is formed.

The thermal death-point is about 56° C. It is pathogenic for mice, less so for guinea-pigs and rabbits. This bacillus is sometimes found in the healthy mouth and nose. It has been known to cause inflammation, especially in the vicinity of the mouth, nose and ear, broncho-pneumonia, and more rarely empyema and meningitis. It was described by Friedländer as the specific cause of lobar pneumonia. Subsequent investigations indicate that it is comparatively seldom found in pneumonia.

There are various capsulated bacilli (capsule bacilli of R. Pfeiffer and others) which closely resemble the bacillus of Friedländer, and at least belong to the same group.

Bacillus of rhinoscleroma.—A short bacillus with rounded ends, often united in pairs, also growing to a greater length; surrounded by a capsule; not motile; stained by the ordinary aniline dyes. Its behavior with Gram's method is said to distinguish it from the bacillus of Friedländer, as the bacillus of rhinoscleroma is not so easily decolorized by Gram's method. This organism has been cultivated. It is aërobic. It grows rapidly, best in the incubator. It does not liquefy gelatin; its growth in gelatin stick-cultures resembles the bacillus of Friedländer. It grows on the ordinary media. Gas may be developed upon potato.

It is pathogenic for mice and guinea-pigs, less so for rabbits. Its virulence is less than that of Friedländer's bacillus.

It has been obtained from the tissues of cases of rhinoscleroma. Rhinoscleroma is a disease characterized by a chronic tubercular thickening and swelling of the skin around the nose and similar swelling of the nasal mucous membrane, sometimes followed by ulceration. It is commonest in Austria and Italy. It has been seen in America only with the greatest rarity.

The organisms may be stained in the diseased tissues, but their detection is a matter of considerable difficulty, and they are not always found. It is not yet certain that they are the cause of rhinoscleroma.

Bacillus pyocyaneus.—A slim bacillus with rounded ends. It is motile. It does not form spores. It is decolorized by Gram's method. It is aërobic; grows well at ordinary temperatures; liquefies gelatin, and grows on the ordinary culture-media. Cultures present a blue or green color, especially in transparent media. This color is not confined to the growth itself, but a blue or green fluorescence spreads over the whole medium. In old agar-cultures the color may become very dark. The pigment forms in the presence of oxygen, and is due, at least in part, to the ptomaine, pyocyanin. On potato the growth is usually brown, which may be tinged with green. Milk is coagulated, and an acid reaction is developed. Indol is formed in Dunham's peptone solution. Blood-serum is liquefied.

The bacillus pyocyaneus seems to be rather widely distributed in nature; it has been found on the skin, in normal feces, also in diarrheal discharges. It is the cause of the color in blue or green pus. It has frequently been demonstrated in pus, but oftenest, perhaps, in mixed infections. It is found in various abscesses, in otitis media, peritonitis, appendicitis and broncho-pneumonia. It is pathogenic for guinea-pigs and rabbits, in whom it may produce septicemia. In animals it may lead only to local suppuration, from which they may recover, being made immune to subsequent infection with this organism. It appears that an antagonism exists between the products of the bacillus pyocyaneus and the anthrax bacillus. Rabbits which have been inoculated with cultures of the anthrax bacillus may recover if they are injected shortly after with a culture of the bacillus pyocyaneus.

Bacillus proteus.—A bacillus with rounded ends, varying much in length, breadth .4 to .6 μ; frequently appearing as short ovals like micrococci; sometimes growing out into long filaments, so that it is said to be pleomorphic. It is motile. Spore formation has not been observed. It is aërobic and facultative anërobic. It grows rapidly at ordinary temperatures. This organism was originally described by Hauser as three different species—*proteus vulgaris*, which was said to liquefy gelatin rapidly, *proteus mirabilis*, which liquefied gelatin slowly, and *proteus Zenkeri*, which did not liquefy gelatin. It seems probable that these organisms were, in fact, varieties of the same species, now called bacillus proteus. Upon gelatin-plates the colonies present a characteristic phenomenon, when seen under the low power, in the projection of processes which subsequently change their form and position, and which may become entirely detached from the original colony, so that the surface of the gelatin may become covered with so-called "swarming islands."

This organism is one of those which were formerly described under the name of bacterium termo. It is among the most common and widely-distributed bacteria. It has been found in decomposing animal and vegetable substances, in the feces, and in the discharges of children having cholera infantum. It appears that this organism may occasionally be pathogenic to man, causing pus-formation, peritonitis, and even general infection. Injection of cultures in considerable amounts may be pathogenic to animals.

Bacillus pyogenes fetidus.—A short bacillus with rounded ends about .5 μ broad and 1.5 μ long; often in pairs; slightly motile; spore formation doubtful. It is aërobic; grows rapidly at ordinary temperatures; does not liquefy gelatin. It grows on ordinary culture-media; the cultures are whitish; upon potato, pale brown; cultures give off a disagreeable odor. It is found in foul pus, often in mixed infections. It probably belongs to the colon group (see Bacillus coli communis).

Bacillus of bubonic plague.—An oval or short rod-shaped bacillus, with rounded ends, sometimes possessing a capsule. It is not motile. It does not form spores. With the aniline dyes the ends stain more deeply than the middle; by Gram's method it appears to be decolorized, but sometimes is said not to be. It is aërobic. Its growths are grayish-white. It does not liquefy blood-serum. It grows best in the incubator, and on most media.

It is killed in four days by drying; in three to four hours by direct sunlight; in a few minutes by steam at 100° C., and in one hour by 1 per cent. carbolic acid. This organism is pathogenic to mice, rats, guinea-pigs, rabbits and sheep, but not to pigeons.

In man it appears usually to enter through wounds in the skin. The Chinese, who are especially prone to the disease, and who go barefooted, are probably very often infected through wounds of the feet. The wound through which the inoculation takes place seems generally to exhibit no inflammatory reaction. The lymph-nodes are greatly swollen, especially the deep inguinal and axillary nodes. The swollen lymph-nodes may suppurate. The suppurating nodes are often infected simultaneously with micrococci. The bacilli are numerous in the enlarged lymph-nodes, but may occur in other organs of the body and in the blood. The period of incubation in this disease is from two to seven days. Although it has not appeared in civilized countries during recent times, it has ravaged the southeastern part of Asia within a few years. In the Middle Ages, and in succeeding centuries, it devastated many of the countries of Europe, where it was one of the most important of the pestilences that went in those times by the name of the "Plague." It appears to have been the disease known in English history as the "Black Death."

Bacillus aërogenes capsulatus.—A thick bacillus, 3 to 6 μ in length, frequently capsulated. The capsules may be found both in cultures and in preparations from animal tissues. It is uncertain whether or not spores are formed. It is not motile. It stains by Gram's method. It is anaërobic, and is readily cultivated by Buchner's method for anaërobes. It grows best at the body temperature, but will grow at the room temperature; does not liquefy gelatin, or but slightly; the growths are whitish. In media containing fermentable substances, as 1 per cent. of dextrose, it produces an abundance of gas. Milk is coagulated, and the reaction becomes acid. Gas forms upon potato, where the growth is thin and grayish-white.

It is not usually pathogenic to rabbits. In guinea-pigs and pigeons it may produce "gas phlegmons." It has been found on numerous occasions in the organs of human cadavers in which a development of gas had taken place, producing bubbles or cavities in the tissues, imparting to them a peculiar spongy character (German, *Schaumorgane*). There is reason to believe that in some cases it enters the body during life. It has been found during life in cases of emphysematous gangrene or cellulitis. This bacillus, or the gas formed by it in the organs of human cadavers, appears to have furnished the basis for some of the cases in which death has been ascribed to the entrance of air into the veins during life. It is probably the organism described by E. Fränkel as the bacillus phlegmones emphysematosæ.

Bacillus edematis maligni (French, *Vibrion septique*).—A bacillus 1 μ in breadth, 3 to 3.5 μ in length, which may form threads, having rounded ends when occurring singly.

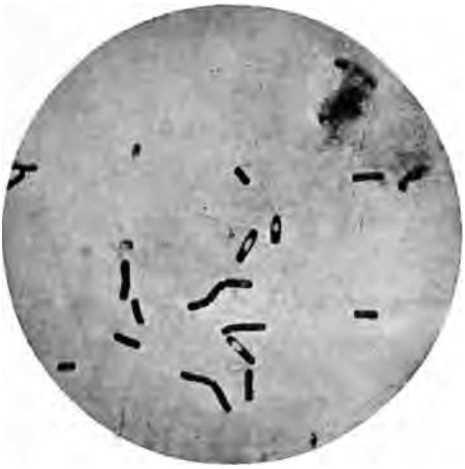


FIG. 57.—Bacillus of malignant edema, showing spores. (Fränkel and Pfeiffer.)

It is motile, having flagella at the sides and ends. It forms spores, and may bulge at the center in consequence of the spores formed there. It is decolorized by Gram's method. It is a strict anaërobe and is best cultivated under hydrogen. It grows at ordinary temperatures, but better in the incubator. It liquefies gelatin and blood-serum. The colonies in gelatin are spherical and appear like little bubbles. It grows well upon agar. Gas may be produced in these media.

It is found in garden earth, street dirt, and in putrefying organic material. It is pathogenic to rabbits, guinea-pigs, mice, pigeons and various other animals, including man. Inoculation results in the production of swelling and edema, spreading from the point of inoculation. Gas may be produced in the tissue. It may lead to widespread septicemia.

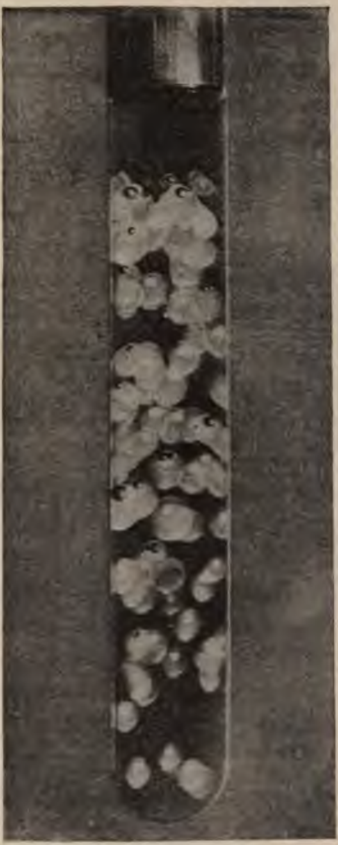


FIG. 58.—Bacillus of malignant edema. Pure culture in dextrose-gelatin. (Fränkel and Pfeiffer.)

Bacillus tetani.—A slim, straight bacillus, with rounded ends, which may form in threads. It is slightly motile. Spores form in culture-media at the end of thirty hours in the incubator. The spores are located at one end, which is swollen, so that in this stage the organism has the shape of a drum-stick. The spores are extremely resistant, and in the dry condition can exist for years. They are killed by moist heat at 100° C. in five minutes; by 5 per cent. carbolic acid in fifteen hours; by bichloride of mercury, 1–1000, in three hours. The tetanus bacillus stains by Gram's method. It is a strict anaërobe; it grows in an atmosphere of hydrogen, but not of carbon dioxide. It may be cultivated at the room temperature, but better in the incubator. It grows upon ordinary culture-media, preferably those containing dextrose. Gelatin is liquefied slowly; the colonies in gelatin present characteristic radiating filaments and look like a thistle. It grows on the other culture-media. Gas formation is not pronounced.

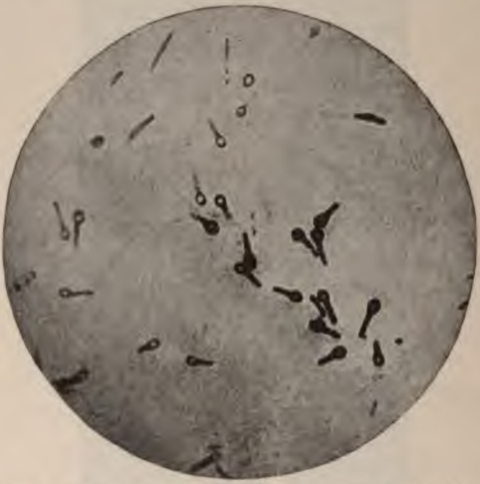
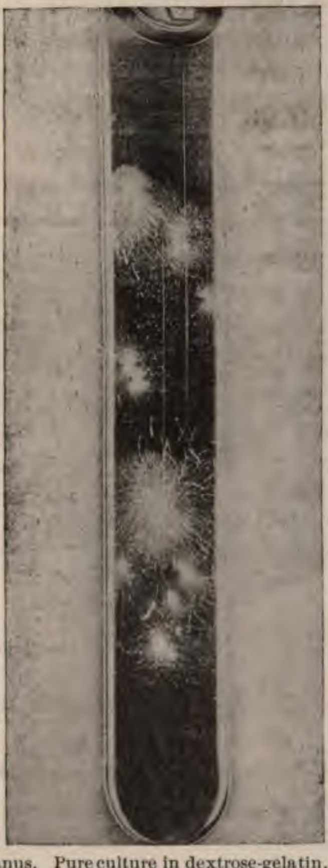


FIG. 59.—Bacillus of tetanus, showing spores. (Fränkel and Pfeiffer.)

'his organism appears to be widely spread in external ıre, especially in the soil. It is often found in garden :h, and in the feces of herbivorous animals. Inoculation



)0.—Bacillus of tetanus. Pure culture in dextrose-gelatin. (Fränkel and Pfeiffer.)

h a pure culture produces tetanus in mice; also in rats, nea-pigs and rabbits. The tetanic spasms begin in the nity of the point of inoculation and afterward become eral. The infection appears almost always, if not always, to be introduced through some wound. The bacilli are not widely scattered through the body; they occur only in the immediate vicinity of the original lesion, and there are no visible alterations in the internal viscera.

Tetanus is the type of the purely toxic disease. Its symptoms may be produced in animals by the injection of liquid cultures which have been deprived of their bacteria by filtration. The toxic substance appears not to be a ptomaine, as was at first supposed, and its exact nature is not determined.

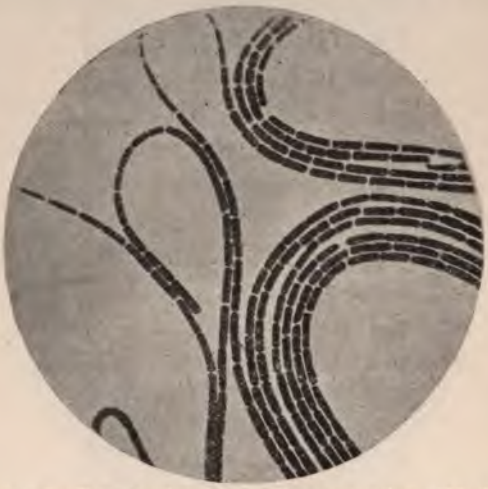


FIG. 61.—Bacillus of anthrax, impression-preparation. (Günther.)

The poison is tremendously powerful (see page 140). The activity of the poison is destroyed by heat, by drying at the body temperature, and by direct sunlight.

Concerning the use of an antitoxin for tetanus, see page 149.

Bacillus anthracis.—This is the largest of the pathogenic bacteria with the exception of the spirillum of relapsing fever, which is longer but more slender. The bacillus of anthrax is 1.5 μ broad, and from 3 to 10 μ long. It often forms long threads. The ends of the bacillus appear slightly concave. It is not motile. It forms spores, which are placed in the centers of the bacilli. The spores form only in the presence of oxygen; they do not appear in the body of an infected animal. Anthrax spores are the most resistant of all pathogenic bacteria; they have been known to withstand boiling for twelve minutes, 5 per cent. carbolic acid for forty days, and 1–1000 bichloride of mercury for nearly three days. The anthrax bacillus is aërobic, although not strictly so. It stains by Gram's method. It grows at the room temperature, but better in the incubator. It liquefies gelatin. Colonies in gelatin seen under a low power display numerous, irregular, fine, hair-like projections; stab-cultures in gelatin also present fine projections passing from the needle-puncture into the solid gelatin. It grows on the ordinary culture-media; the growths are usually whitish. Cultures on potato kept in the incubator are particularly favorable to the development of spores.

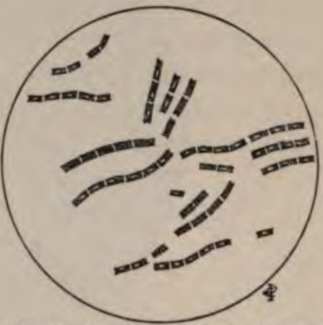


FIG. 62.—Bacillus of anthrax showing spores, drawn from a cover-glass preparation made from a pure culture.

It is pathogenic to mice and guinea-pigs, less so to rabbits; it is also pathogenic to sheep and cattle. Rats and pigeons are quite resistant but not entirely immune; pigs, dogs and most birds are not susceptible.

Anthrax is a disease which occurs chiefly in cattle and sheep. It is commoner on the continent of Europe and in Siberia than in America. In susceptible animals inoculated with virulent cultures of the anthrax bacillus it produces septicemia. Large numbers of the bacilli are found in the blood, and may be crowded together in the capillaries of the liver and kidneys. Men are occasionally affected, especially those whose occupations bring them in contact with cattle or with the hides and wool of animals that die of the

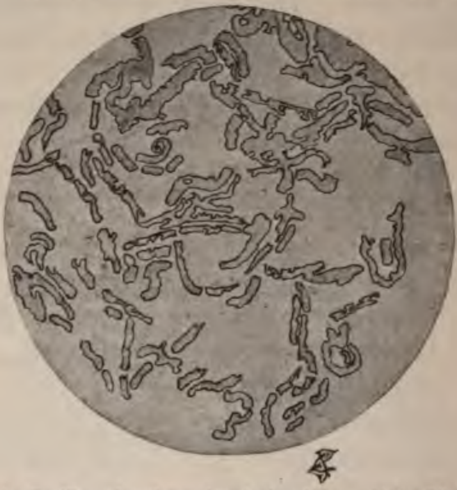


FIG. 63.—Bacillus of anthrax, involution forms. (After Günther.)

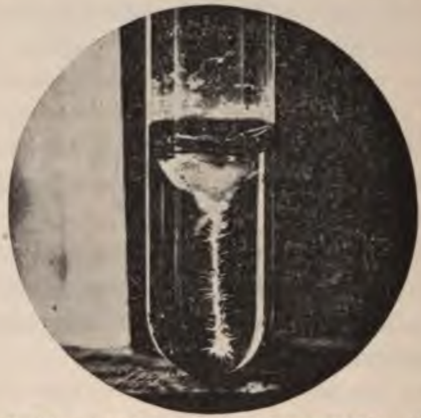


FIG. 64.—Bacillus of anthrax. Stick-culture in gelatin. (Günther.)

disease. The infection may enter through wounds of the skin, where it usually produces a localized inflammation known as malignant pustule. Anthrax of the lungs may be acquired by inhalation of material containing the spores of the bacilli ("Wool-sorter's disease"). Infection by way of the intestine occurs occasionally but is less common.

The anthrax bacillus, owing to its large size, was the first of the pathogenic bacteria to be recognized, and its study has furnished the basis of much of our knowledge concerning the infectious diseases. It was for anthrax that Pasteur developed the idea of making a protective vaccine, shortly after he had invented a similar vaccine for chicken-cholera.

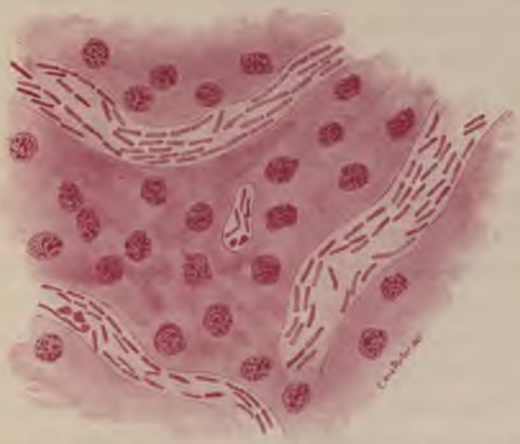


FIG. 65.—Bacillus of anthrax in the capillaries of the liver of a mouse, from a section stained with fuchsin.

In order to obtain material free from spores the blood of an animal which has recently died of anthrax is taken, because anthrax spores do not form in the animal body. Cultures made in bouillon are kept at a temperature of from 42° to 43° C. At this temperature spores do not form, while the virulence of the anthrax bacillus becomes gradually diminished. In time the virulence is so far diminished that rabbits will survive inoculation, and eventually also mice and guinea-pigs, which are extremely susceptible to anthrax. Small doses of a culture of extremely weak virulence are given to the animals which it is desired to protect, like cattle and sheep, and subsequently a somewhat more virulent culture is employed.

Bacillus of influenza.—A small bacillus with rounded ends. It does not form spores, is not motile, and is decolorized by Gram's method. It is aërobic, grows only in the incubator, and upon media containing blood or leucocytes. The medium is prepared by smearing sterile blood over the surface of a tube of agar. The colonies are small and transparent, looking like little drops of water, not becoming confluent.

Out of a large number of bacilli, most of them are destroyed in twenty-four hours or less by drying. They die out in a similar manner in water. Experiments upon animals appear up to this time not to have been very convincing. As far as is known, this organism grows only in man, and not outside of the human body. In cases of influenza it is found in the mucous discharges, and in the bronchi and lungs. According to Canon, the bacilli may sometimes be found in the blood.

Bacillus diphtheriæ (Klebs-Löffler). — A straight or slightly-curved bacillus, usually 1.2 to 2.5 μ in length, with rounded or slightly pointed ends, remarkable for showing irregularities of form, sometimes being club-shaped or spindle-shaped; branching forms are said to have been found, but they must occur with very great rarity. It is not motile, and does not form spores. It retains its color after Gram's method, but it is best stained with watery solutions of the aniline dyes, especially Löffler's alkaline methylene-blue.

It is peculiar in staining irregularly; certain spots stain more sharply than other portions, and darkly-stained spots are likely to occur at the ends. It is aërobic and facultative anaërobic. It grows most rapidly in the incubator, and slowly, or not at all, below 20° C. Gelatin is not liquefied. It may be cultivated on various alkaline culture-media, but grows best on Löffler's blood-serum mixture. On this medium the growth consists of small white or cream-colored, slightly elevated colonies, which may become confluent. The morphology of the bacillus is most characteristic when it is cultivated on blood-serum. It also grows upon glycerin-agar. On potato it produces an invisible growth (see Bacillus of typhoid fever). In alkaline bouillon containing a carbohydrate the reaction becomes acid in forty-eight hours. The reaction of the bouillon subsequently becomes alkaline. The growth may form a pellicle over the surface of the bouillon. It has also been successfully cultivated on various media to which egg-albumen has been added.

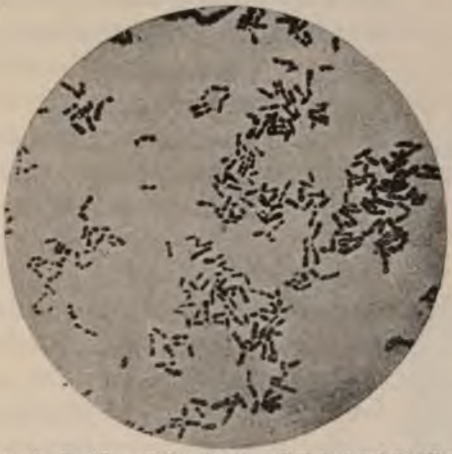


FIG. 66.—Bacillus of diphtheria. (Fränkel and Pfeiffer.)

It is killed by a temperature of 58° C. in ten minutes. It resists desiccation well.

Bacteriological diagnosis of Diphtheria.—In many large cities the bacteriological diagnosis of diphtheria is undertaken by boards of health. The methods used differ somewhat in detail, but are similar in the main, and are based upon the procedure devised by Biggs and Park for the Board of Health of New York City. Two tubes are furnished in a box. The tubes are like ordinary test-tubes, about three inches in length, rather heavy, and without a flange. Both are plugged with cotton. One contains slanted and sterilized Löffler's blood-serum mixture; the other contains a steel rod, around the lower end of which a pledget of absorbent cotton has been wound and the tube afterward sterilized. Instead of this rod wrapped with cotton, a heavy wire loop inserted into a handle is sometimes employed. The swab is wiped over the suspected region in the throat, taking care that it touches nothing else, and is then rubbed over the surface of the blood-serum mixture. The swab is returned to its test-tube and the cotton plugs are returned. to their respective tubes. The plugs, of course, are held in the fingers during the operation, and care must be taken that the portion of the plug that goes into the tube touches neither the finger nor any other object. The principles, in fact, are the same as those laid down in general for the inoculation of culture-tubes with bacteria (see page 62). In board of health work these tubes are returned to the office. When it is desirable, a second tube may be inoculated from the swab. The tubes are placed in the incubator, where they remain for from twelve to twenty-four hours, and a microscopical examination is

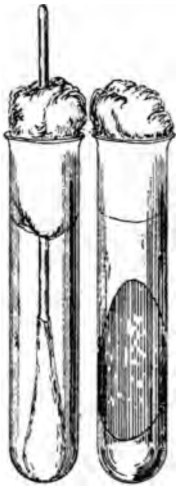


FIG. 67.—Swab and culture-tube used in the diagnosis of diphtheria.

then made of smear-preparations stained with Löffler's methylene-blue. On Löffler's blood-serum kept in the incubator the bacillus of diphtheria grows more rapidly than the other organisms which are ordinarily encountered in the throat, a property which to a certain extent sifts it out, as it were, from them, and makes its recognition with the microscope easy in most cases. The growth, furthermore, is quite characteristic, and its nature can be predicted with considerable accuracy, even without microscopical examination, by one who has had much practice. Colonies of streptococci frequently look very like those of the bacillus of diphtheria, but these two are easily distinguished from each other with the microscope. The diagnosis of the diphtheria bacillus, then, is made from the character of the growth upon blood-serum and the microscopical examination, taking into account the size and shape of the bacilli, with the frequent occurrence of irregular forms and the peculiar irregularities in staining.

The very large number of examinations, that have been made by various boards of health, have shown that pseudomembranous inflammations of the throat are sometimes caused by streptococci alone. They have also shown that the diphtheria bacillus may persist in the throat for a long time, occasionally several weeks after the patient has apparently recovered; also that diphtheria bacilli are occasionally found in the throat when there is an inflammatory condition without any pseudo-membrane, and that they sometimes appear in an apparently healthy throat, especially in children who have been associated with cases of diphtheria. It has been found that bacilli sometimes occur in the throat which have all the morphological and cultural properties of the diphtheria bacillus, but which are devoid of virulence when tested upon animals. Such diphtheria bacilli have sometimes been called pseudo-diphtheria bacilli. American writers are disposed to regard them as being genuine diphtheria bacilli whose virulence has become attenuated, and prefer to give the name *pseudo-diphtheria bacillus* to an organism which resembles the genuine diphtheria bacillus in some respects, but having distinct morphological and cultural peculiarities of its own which enable it to be recognized.

"The name pseudo-diphtheria bacillus should be confined to bacilli which, although resembling the diphtheria bacillus, differ from it not only by absence of virulence, but also by cultural peculiarities, the most important of the latter being greater luxuriance of growth on agar and the preservation of the alkaline reaction of bouillon cultures. The pseudo-diphtheria bacillus may render bouillon cultures acid in forty-eight hours when grown anaërobically. The pseudo-diphtheria bacillus in this sense was found in a number of cases, but not frequently. It is probably of different species from the genuine diphtheria bacillus, and is without diagnostic importance." (WELCH.)

The diphtheria bacillus is pathogenic to animals. When it is injected into them it produces a toxemia. In the guinea-pig, which is especially susceptible, local inflammation results, and death occurs usually in two or three days. The bacilli are found to be confined to the vicinity of the wound, and not usually to be disseminated throughout the whole body. The death of the animal, therefore, is due to the poisons elaborated by the diphtheria bacilli—either poisons introduced at the original injection, or substances produced by the bacilli which may have multiplied in the animal's body. The internal viscera, especially the liver, often exhibit small areas consisting of necrotic cells; a transudation of serum takes place in the great serous cavities, and the lymph-nodes are swollen. A genuine diphtheritic membrane may be produced on the trachea of a young kitten by rubbing into it a part of a culture of the diphtheria bacillus.

As is well known, the pseudo-membranous affection produced by the diphtheria bacillus in man is generally seen in the larynx and pharynx. Membranous rhinitis is also caused by the diphtheria bacillus. On the other hand, pseudo-membranous affections of the larynx and pharynx may be produced by streptococci. Pseudo-membranes occurring in the throat during scarlet fever and measles may be due to the diphtheria bacillus, but are more often caused by streptococci. The affection known as membranous croup is usually diphtheria of the larynx, produced by the diphtheria bacillus. Although the uninjured skin is not attacked by the diphtheria bacillus, it may be present in pseudo-membranes on wounded surfaces, usually in connection with diphtheria in the throat. Most pseudo-membranes formed upon wounds of the skin are produced by other bacteria than the diphtheria bacillus, as is also the case with the pseudo-membranous inflammations of the intestines and bladder. Although such inflammations are often called "diphtheritic," it must be remembered that the expression is used in an anatomical sense, meaning that a fibrinous pseudo-membrane has formed, extending deeply into the tissues, which is not necessarily caused by the diphtheria bacillus.

In cases of diphtheria in man, the diphtheria bacillus is generally found limited to the vicinity of the pseudo-membrane, and at autopsies it has rarely been found in the internal viscera, excepting in the lungs, where diphtheria bacilli may or may not be present when diphtheria is complicated with broncho-pneumonia. The general symptoms of the disease, including the paralysis which sometimes follows it, are due to the toxins produced by the bacilli in the throat.

Concerning the use of antitoxins in diphtheria, see page 148.

Bacillus tuberculosis.—A slim bacillus 1.5 to 4 μ in length, which very frequently presents a beaded appearance, owing to its being dotted with bright, shining spots. Branching forms have been described, but they certainly occur very rarely. It is not motile. It has not been proved that spores are formed; nevertheless certain structures, like caseous lymph-nodes, have been shown to be capable of infecting guinea-pigs with tuberculosis, although tubercle bacilli could not be demonstrated in them with the microscope. This makes it seem possible that the organisms were present as spores which eluded the microscopical examination. The tubercle bacilli stain with the ordinary aniline dyes and by Gram's method. As has already been stated, when stained with aniline-water dyes or carbol-fuchsin they are not readily decolorized by acids and alcohol, which fact distinguishes them from all other known bacteria excepting the leprosy bacillus, the bacilli of smegma, and possibly the bacillus of syphilis (Lustgarten). (See pages 29 and 122.) According to recent researches of Koch, the tubercle bacilli owe their peculiar staining proper-

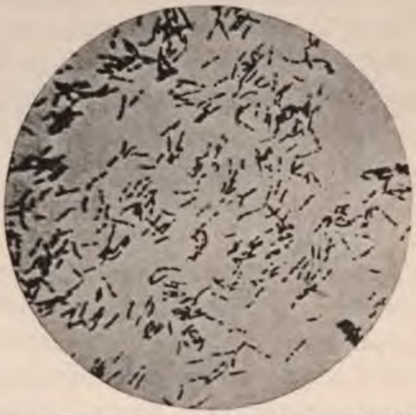


FIG. 68.—Bacillus tuberculosis, from a pure culture. (Fränkel and Pfeiffer.)

ties to fatty acids contained in the bodies of the bacilli. In stained preparations the bacillus usually appears very distinctly beaded, owing to the presence of stained areas which alternate with unstained areas; these unstained areas are considered by some to be spores.

The bacillus tuberculosis is aërobic and facultative anaërobic. It is cultivated with considerable difficulty, best at about 38° C. It does not grow at a temperature below 29° C., and cannot therefore be cultivated upon gelatin. It grows best upon blood-serum, where the growth becomes visible in from ten to fourteen days in the incubator. It forms a dry, mealy, scaly mass, elevated above the surface, of a grayish-brown color. It also grows upon glycerin-agar; or glycerin-bouillon, on which it forms a pellicle; upon potato; and upon milk containing 1 per cent. of agar. It can be cultivated from tuberculous sputum only with very great difficulty. It is best to obtain it from the tissues of an animal that has died of tuberculosis, where the tubercle bacilli may be found unmixed with other bacteria. Pieces of tissue should be taken with the precautions necessary to avoid contamination, and should be broken up and rubbed over the surface of the medium. The tubes must be closed with sealing-wax or covered with rubber caps, to prevent

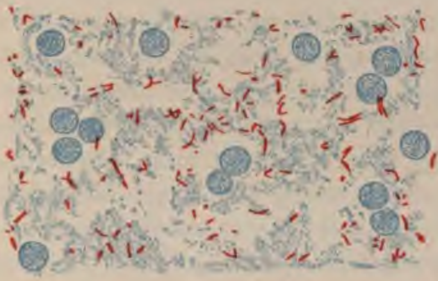


FIG. 69.—Bacillus tuberculosis in sputum, stained with fuchsin and methylene-blue. (von Jaksch.)

drying in the incubator (Fig. 23). If rubber caps are used they should first be left in 1–1000 bichloride of mercury for an hour, and the cotton plug should be burned before putting on the rubber cap. A number of tubes should be inoculated, using rather large particles of the tuberculous material. Among the tubes inoculated, many will fail to present any growth. After the organism has once been grown upon a culture-medium it may be propagated with less difficulty. It is best cultivated the first time upon blood-serum.

It is killed by 5 per cent. solution of carbolic acid in a few minutes. In sputum it is destroyed in twenty-four hours by a 3 per cent. solution of carbolic acid. It resists desiccation for months, but is killed in some hours by direct sunlight. It is destroyed in a few minutes by boiling.

It is not known to grow outside of the animal body. It is the cause of tuberculosis in man. It produces tuberculosis in apes, cows, sheep, horses, rabbits, guinea-pigs, cats, field-mice, and occasionally in other animals. Guinea-pigs and rabbits are extremely susceptible. A guinea-pig inoculated with tuberculous sputum (provided it does not die of septicemia, due to the pyogenic micrococci which are frequently present in sputum) will present a swelling of the neighboring lymph-nodes in the course of two to four weeks, and will die as a rule in from four to eight weeks, although the time may be longer.

The lesion produced by the tubercle bacilli in the tissues of men and the lower animals is called a tubercle, which in the beginning is a grayish-white area about the size of a millet-seed. In sections of the tissue young tubercles are found to present several different structures. Near the center, one or more very large cells called giant-cells occur. They contain several or many nuclei which are frequently arranged in a crescentic manner at one side of the cell. Tubercle bacilli can often be demonstrated inside of the giant-cell. Except possibly in the very youngest tubercles, a small area of necrotic tissue will always be found at the center of the tubercle.

Around the giant-cells and the necrotic area are seen large cells with distinct nuclei which resemble epithelial cells, and are often called epithelioid cells; they are also often termed granulation cells, and represent an attempt at the formation of granulation tissue. But no new-formed blood-vessels, such as are found in granulation tissue as a rule, occur in the tubercle. Tubercle bacilli may also be found among the epithelioid cells. Outside of these epithelioid cells is another layer of small cells called lymphoid cells which represent leucocytes that have appeared in this situation as a part of the inflammatory reaction excited by the presence of the tubercles. The zone of lymphoid cells may be very indistinct or wanting. Frequently it may be very difficult to make out that the cells are arranged in distinct zones at all. The cells are imbedded in a matrix consisting of the connective tissue originally belonging to the part, to which some fibrin may be added. In addition to the fact that no new blood-vessels are formed to maintain the nutrition of these newly-formed cells, the small vessels included in the tubercle and around it suffer from inflammatory changes. Owing to these causes and to a toxic substance formed by or in the tubercle bacilli, degenerative changes and necrosis take place at the central part of the tubercle. As a result of these degenerative changes the center of the tubercle becomes converted into a dry, yellowish-white, friable mass, resembling dry cream-cheese. Such material is said to be caseous, and the process is called *caseation*. Prudden and Hodenpyl found that the injection of dead tubercle bacilli into animals produced lesions having the histological characters of tubercles, but caseation did not take place.

The small tubercles first formed are called *gray* or *miliary tubercles.* As they become larger they also frequently become confluent. The larger, confluent, caseous tubercles are often called *yellow tubercles.* Swollen tuberculous lymph-nodes of the neck are among the manifestations of the condition formerly known as *scrofula.*

Very frequently, especially in the lungs, miliary tubercles are not formed at all, but a kind of diffuse tubercle tissue, in which caseation also takes place. The caseous masses often undergo softening. In the lungs the discharge of the softened material results in the formation of a cavity. This formation of a cavity in the lungs is frequently, if not usually, accompanied by secondary infection with pyogenic micrococci. Caseous tuberculous masses often become partly calcified. Very often they may be encapsulated by new formed fibrous or scar tissue. It is possible for tuberculosis to become cured to all practical purposes by means of this process. Autopsies on human subjects have shown that such cures very often take place, especially in tuberculosis of the lungs occurring over a localized area. When a tuberculous area has become caseous and encapsulated, and apparently quiescent, it is possible for it to be excited to renewed activity under suitable conditions, and, owing to the softening and the discharge of infected material into one of the vessels or cavities of the body, a wide-spreading and rapidly fatal tuberculosis may follow.

Tuberculosis may become disseminated throughout the body from a small focus as a starting-point. The tubercle bacilli may travel through the lymph-spaces and affect adjacent tissues, some of them reaching the nearest group of lymph-nodes. In tuberculosis of the lungs it is usual also to find tubercles in the bronchial lymph-nodes, and in tuberculosis of the intestines there is also tuberculosis of the mesenteric lymph-nodes. The disease may travel along the serous surfaces and become widely scattered throughout a cavity like that of the pleura or peritoneum. The bacilli may be expelled on some mucous surface and be carried along it to infect some point farther on, as happens when the larynx becomes infected in tuberculosis of the lungs, and when in the same disease tuberculous sputum is swallowed and leads to infection of the intestines. Finally, the infected material may enter the blood-vessels and be swept along with the blood-current to become scattered generally throughout the body. In such cases we are likely to have general or *acute miliary tuberculosis.* Almost every organ of the human body may be infected by tuberculosis. Among the most common may be mentioned the lungs, the lymph-nodes, the bones, the intestines, the skin, the meninges, and the serous membranes.

Infection, as far as we know, is always to be attributed directly or indirectly to some preëxisting case of tuberculosis in man or the lower animals. The entrance into the body is most commonly by way of the lungs, where also tuberculous disease is commonest in man, going by the name of *consumption.* This is doubtless due to the prevalent habit of expectorating in public places. Out of fifty-six samples of sputum collected in street cars by Dr. W. G. Bissell, City Bacteriologist in Buffalo, four were tuberculous. In forty-eight samples taken from the floors of a public building by Dr. C. R. Orr, of the pathological laboratory of the University of Buffalo, tubercle bacilli were found three times. According to the researches of Nuttall, a case of tuberculosis may expectorate many millions of tubercle bacilli in the course of twenty-four hours.

Concerning the occurrence of tubercle bacilli in cow's milk, see pages 117 and 119.

Cases have been recorded in which the disease was transmitted from the mother to the child in the uterus; how fre-

[illegible] has been [illegible] that some [illegible] tubercle bacilli [illegible] cultures [illegible] porcelain. Koch [illegible] tuberculin called [illegible] extract made from [illegible] bacilli. The value of [illegible] at least doubtful, and [illegible] with a great deal of

Tuberculosis of Birds. Fowls, ducks and other birds sometimes suffer from tuberculosis due to a bacillus closely resembling the tubercle bacillus of mammals. It has similar staining properties. It sometimes grows in long, branchng forms. It differs somewhat from the tubercle bacilli of mammals in its cultural properties. The liver is the organ most often affected. Guinea-pigs are much less susceptible o it than to mammalian tuberculosis. Rabbits are somewhat susceptible, though less so than to mammalian tuberculosis.

Pseudo-tuberculosis.—Guinea-pigs and other rodents sometimes present lesions macroscopically very similar to those of tuberculosis, in which, however, the tubercle bacilli cannot be found. The affection appears not to be tuberculosis at all, and its nature is not well understood. A specific bacillus has been described as the cause of this disease which is entirely unlike the tubercle bacillus.

Bacillus lepræ (of leprosy).—A slim bacillus from 4 to 6 μ in length. It is probably not motile. It is uncertain whether or not it forms spores. It stains by the Gram and the Weigert fibrin method, and it is also colored by the methods used for staining the tubercle bacillus. It takes the dye, however, more readily than the tubercle bacillus. In stained preparations it appears very similar to the tubercle bacillus, and resembles it in having alternate colored and unstained spots. Although several observers have reported success in attempts to cultivate the bacillus of leprosy, their claims have been disputed. The results of inoculation into man and the lower animals of material coming from cases of leprosy have also been uncertain. The bacillus of leprosy has been found so constantly in the tissues of those having the disease that it is generally admitted to be the specific cause. The skin and the peripheral nerves are the parts most affected, although other tissues and the internal viscera may be involved. A granulation tissue, forming nodules and thickenings, appears in the affected parts. The bacilli are found in large numbers in the nodules, partly outside of the cells, but mostly within the cells. It is still uncertain whether or not the disease can be transmitted directly from one individual to another, in extrauterine life, or whether it can be inherited from the parents.

Bacillus mallei (of glanders).—A slim bacillus with round or pointed ends, which often shows alternate light and dark spots in stained preparations. It is not motile. It probably does not form spores. It is decolorized by Gram's method. After staining with the ordinary aniline dyes it is easily decolorized, and on that account it is difficult to demonstrate in sections of tissues. It is aërobic and facultative anërobic. It grows at the room temperature, but better in the incubator. It grows slowly on gelatin, and does not liquefy it, or only after a long time. On agar it produces a moist, white growth; on blood-serum a yellowish or brownish growth; blood-serum is not liquefied. Milk is coagulated slowly, and the reaction becomes acid. On potato the growth is characteristic in one or two days

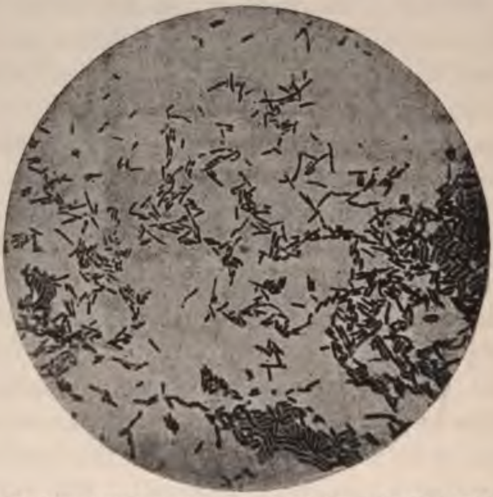


FIG. 70.—Bacillus of glanders, from a pure culture. (Fränkel and Pfeiffer.)

in the incubator, becoming translucent amber-yellow, later a reddish-brown, while the surface of the potato becomes discolored.

It is killed in five minutes by a 5 per cent. solution of carbolic acid, in two minutes by 1–5000 bichloride of mercury, in ten minutes by a temperature of 55° C. It may survive drying for a number of weeks.

In the horse and ass it produces the disease known as glanders, which affects the mucous membrane of the nasal cavity. When the skin is involved the disease goes by the name of farcy. In the nose, nodules appear in the mucous membrane which become necrotic, forming ulcers. They may become confluent, and may extend along the adjacent surfaces as far as the lungs. There is a profuse discharge from the nose. The neighboring lymph-nodes become involved and are swollen, and nodules may be present in the internal viscera. In the skin the nodes lying underneath the skin are called farcy-buds. Histologically the nodules consist of a granulation tissue, but they tend to break down rapidly, and the process in some respects is very like ordinary suppuration.

This bacillus is pathogenic to guinea-pigs, field-mice and cats; rabbits and dogs are less susceptible or only slightly so; white and house-mice, rats, cattle and hogs are immune. Men are occasionally affected, especially those who have come in contact with horses. The mucous membrane of the nasal cavity may be the part involved, or the skin, or the internal viscera.

The diagnosis of the disease is best effected by the inoculation of a male guinea-pig with the material from a case suspected of being glanders, introducing it into the peritoneal cavity. In about two to five days there appears a characteristic swelling of the testicle indicating the beginning of suppuration, which presently takes place; the animal usually dies after two or more weeks.

Mallein is a product obtained from an old glycerin-bouillon culture of the bacillus mallei. The cultures are placed in a steam sterilizer for several hours, and are filtered through unglazed porcelain. The filtrate contains the products of the growth of the bacillus mallei and is of much the same character as tuberculin. Injected into animals suspected of having glanders, if it produces a local and febrile reaction, the existence of glanders is indicated. It is usually successful in the diagnosis of the disease in lower animals, especially in horses, where it has been largely employed.

Actinomyces (Streptothrix actinomyces, Ray-fungus of actinomycosis).—The morphology of this organism is quite different from that of most of the bacteria. It is generally considered to be a bacterium of a higher type, related to cladothrix, or a form of fungus higher than the bacteria. The organism appears in the form of threads which branch dichotomously. These threads make radiating, interlacing masses, and their external ends are swollen and bulbous. Colonies formed in this manner, seen under moderate magnification, have a radiating appearance which has given rise to the name, ray-fungus. The club-shaped external ends are readily distinguished and the growth possesses a very distinctive form. This is the shape which the organism presents as it grows in the animal body. The club-shaped ends are generally regarded as a degenerative or involution form. Transverse divisions may be distinguished upon the threads. Spherical forms resembling micrococci may appear which may possibly be spores. The organism stains with the ordinary aniline dyes, by Gram's method or the Weigert fibrin stain.

The fungus may be cultivated upon agar. The growth is not rapid. The colonies are fine, dry, elevated, irregular in form, becoming opaque. Bulbous ends upon the threads do not usually appear in cultures. The results of the injection of these cultures into the lower animals are as yet uncertain.

The disease produced by the ray-fungus is called actinomycosis. It occurs in cattle, swine, horses, and occasionally in man. Infection appears to be carried by grain or particles of vegetable fiber which penetrate the tissue. The infectious material frequently enters through the mouth, especially in the vicinity of the teeth, but it may also occur through the skin or the mucous membranes. It leads to the formation of inflammatory, tumor-like nodules, hence the name "lump-jaw" given to the disease in cattle. Necrosis of the tissue takes place with the formation of an abscess. The pus is peculiar in containing small whitish particles which consist of little colonies of the ray-fungus, and which readily permit the disease to be diagnosed by the microscope. The material may be examined in the perfectly fresh condition without any staining. The jaw or its neighborhood is very frequently affected, or the disease may be present in other situations about the head and neck, and may involve the lungs, the intestines, and the vertebræ, ribs, and other bones. The disease is usually localized, but a number of areas may be affected simultaneously.

Madura disease, Madura foot, or mycetoma, is a disease occurring in India affecting one of the extremities, characterized by swellings, nodular deposits and abscesses. It is due to a fungus similar to or identical with the ray-fungus of actinomycosis. There appear to be several varieties or species nearly related to actinomyces.

Bacillus typhi abdominalis (Bacillus of Eberth).—A bacillus with rounded ends, varying in length, sometimes making very short, oval forms, sometimes growing out into long threads. It is very actively motile, and possesses numerous flagella which arise from all parts of the surface. It probably does not form spores. It is not stained by Gram's method, but it may be colored with the ordinary aniline dyes, when the stain will frequently be somewhat irregular. It may be stained in sections of tissues from cases of typhoid fever, with the aniline dyes, such as Löffler's alkaline methylene-blue. It is aërobic and facultative anaërobic. It grows at ordinary temperatures, better in the incubator, but grows rather slowly. Gelatin is not liquefied. Young surface colonies in gelatin appear whitish, with irregular borders and more or less wrinkled surfaces. It grows on the ordinary media, and the growths are whitish. Bouillon is clouded. Milk becomes slightly acid, but is not coagulated. In media containing dextrose acid is formed, but no gas. In lactose-bouillon neither acid nor gas is formed, although when grown in milk the typhoid bacilli produce an acid reaction. The lactose-litmus-

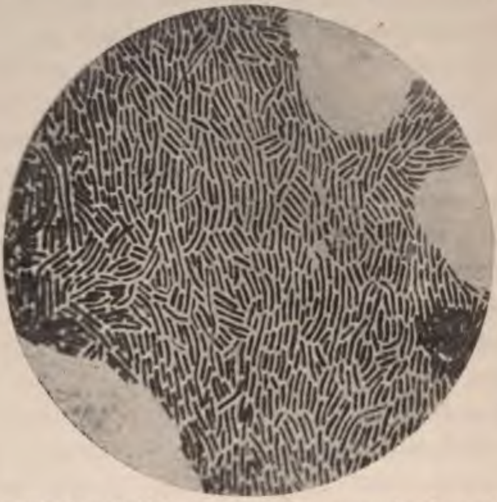


FIG. 71.—Bacillus of typhoid fever. Impression-preparation from a colony on a gelatin-plate. (Fränkel and Pfeiffer.)

gelatin or agar of Wurtz makes use of the blue tinge possessed by colonies of the typhoid bacillus on this medium to distinguish them from colonies of the colon bacillus and other bacteria which form acids from lactose. In Dunham's peptone solution indol is not formed, as a rule. On potato it usually forms what is called an invisible growth; that is, although no development is apparent to the eye, numerous bacilli may be shown under the microscope in smear preparations made from the surface of potato inoculated about forty-eight hours previously. Occasionally a slight visible growth is seen on potato.

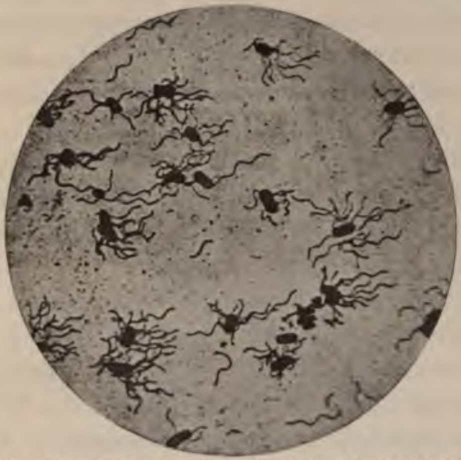


FIG. 72.—Bacillus of typhoid fever, stained by Löffler's method to show flagella. (Fränkel and Pfeiffer.)

The typhoid bacillus is killed at 60° C. in ten minutes.

Upon the medium of Elsner (see page 59), which has been recommended for the isolation of the typhoid bacillus from substances containing a mixture of bacteria, especially feces and water, the development of the typhoid and colon bacilli is favored, while that of the other bacteria is more or less inhibited; the colonies of the typhoid and colon bacilli are easily recognized under the low power of the microscope.

A new medium has recently been suggested by Hiss for the isolation of the typhoid bacillus. It consists of gelatin and agar, beef-extract, sodium chloride and dextrose, and is given a slightly acid reaction. These substances are used in different proportions for plate- and for tube-cultures. This medium is of a semi-solid character, and makes use of the great motility of the typhoid bacillus in producing a uniform clouding of the medium in tubes, with the absence of gas formation; while in plate-cultures the colonies exhibit peculiar filamentous outgrowths. It is claimed that it can be determined whether organisms are typhoid bacilli or not after thirty-six hours in the incubator.

M. W. Richardson has recently devised an application of the "Widal" serum-test to plate-colonies suspected of containing typhoid bacilli. If a typhoid colony be torn with a needle, under moderate magnification "a seething motion resembling much the appearance of a swarm of bees" may be seen. This appearance is due to the motility of the bacteria. If such a colony be touched with a small quantity of blood-serum from a case of typhoid fever, the motion is said to cease instantly and almost absolutely. Colonies of other motile bacteria do not undergo a corresponding loss of motility.

For a comparison of the properties of the bacillus of typhoid fever and the bacillus coli communis, see the latter.

Concerning the detection of the bacillus of typhoid fever in water, see page 113.

THE WIDAL SERUM-TEST FOR TYPHOID FEVER.

When a small quantity of a culture of typhoid bacilli is mixed with a little blood-serum derived from a case of typhoid fever, within a few minutes the motility of the typhoid bacilli is abolished and they become agglutinated into clumps or masses. This reaction does not take place with the blood-serum of healthy persons or of those suffering with other diseases, nor when the blood-serum of a typhoid fever case is mixed with motile bacteria other than typhoid bacilli. It has been observed in the blood-serum of an infant born while the mother was convalescing from typhoid fever.

The agglutinating substance has been found in blister-serum and in the milk of typhoid cases, in fluids from the serous cavities and inflammatory and edematous areas in variable amounts, and occasionally in urine, bile and tears.

The reaction may be obtained by adding blood-serum to a young bouillon-culture of typhoid bacilli kept in the incubator, when the occurrence of agglutination becomes manifest by the collection of the bacteria into visible masses or flocculi, which form a sediment. Most investigators prefer to watch the results under the microscope, using an ordinary slide, or, better, the hanging-drop. Young cultures—less than twenty-four hours old—in bouillon, and kept in the incubator, are to be used. Johnston and McTaggart recommend cultures of attenuated virulence for use with the dry-blood method (see below). Cultures on agar, also freshly made, may be employed, mixing a little of the growth with bouillon at the time of using.

Blood-serum, blister-serum, fresh blood and dried blood have all been tried with success. Blood dried on paper or cover-glasses as proposed by Wyatt Johnston is extremely convenient. To perform the test, it is mixed with sterilized distilled water; the objection to it lies in the difficulty of securing an accurate dilution. The New York Board of Health have found blister-serum satisfactory and easy to obtain. A little of the serum is mixed on the cover-glass with a definite amount of the fresh bouillon-culture, and is [illegible] In a short time the chara- [illegible] motility occurs. At the same [illegible] and [illegible] a drop of the culture [illegible] similarly diluted, should be ex- [illegible] used vary from 1 part [illegible] The time within which the re- [illegible] minutes to one or two [illegible] time should be shorter [illegible] Both clumping and [illegible] take place. In a positive case [illegible] Normal blood sometimes [illegible] some degree. If the [illegible] should be tried [illegible] the result should be [illegible] typhoid fever.

[illegible] the seventh day of [illegible] is often delayed and [illegible] be repeated when

[illegible] to typhoid fever [illegible] in 95.2 [illegible] for which

[illegible] after recovery. [illegible] it may [illegible] borne in

[illegible] from the [illegible] only with [illegible] and others [illegible] they are best [illegible] it is to be [illegible] may also be present. Puncture of the spleen with a sterilized hypodermic needle, during life, has also been resorted to as a means of diagnosis. The drop of fluid withdrawn may be examined by culture-methods for typhoid bacilli. There is probably some danger to the patient attending this procedure. Typhoid bacilli have been found in urine and the examination of urine for them has been used in diagnosis.

Inoculation experiments in animals have not been very satisfactory. With a few exceptions, possibly, anatomical lesions resembling those of typhoid fever have not been produced by the inoculation of typhoid bacilli into animals. The injection of cultures into animals may produce death, but it can usually be shown to result from the poisons contained in the cultures.

Typhoid fever is rare during the first two years of life. It frequently attacks young and robust men. The causes that bring about susceptibility to infection are not known.

The principal lesion in typhoid fever lies in the Peyer's patches of the lower part of the small intestines; the mesenteric lymph-nodes and spleen also are swollen. The typhoid bacillus may be demonstrated in sections of the walls of the diseased portions of the intestine. Cases are recorded in which no lesions were found in the intestines but where the typhoid bacilli were widely spread through the organs of the body, and which therefore represented typhoid septicemia.

Periostitis and osteomyelitis, which are not uncommon sequellæ of typhoid fever, may be caused by typhoid bacilli. Ordinary suppuration may be produced by the typhoid bacillus, but most suppurative affections during or following typhoid fever are mixed infections, or are due to the ordinary pyogenic bacteria.

Bacillus coli communis (Bacterium coli commune of Escherich, probably the same as Bacillus Neapolitanus of

Emmerich, often called simply the colon bacillus).—A bacillus with rounded ends, frequently of a short, oval form, when it may be difficult to distinguish from micrococci; often longer; often forming threads. It is slightly motile, having several flagella. It does not form spores. It stains with the ordinary aniline dyes and is decolorized by Gram's method. It is aërobic and facultative anaërobic. It grows well at the room temperature, but more rapidly in the incubator. It does not liquefy gelatin. In gelatin-plates the surface colonies are of a bluish-white color; the centers are denser than the borders, which are translucent. It usually grows more rapidly in gelatin than the bacillus of typhoid fever. Its growths in other media are mostly whitish. Bouillon becomes clouded. In peptone solution it forms indol. On potato it forms an abundant visible growth, from cream-color to brown. Milk becomes acid and is usually, but not always, coagulated slowly. It causes the development of gas and acid in media containing dextrose, lactose, or saccharose. Differential points between the bacillus of typhoid fever and the bacillus coli communis are as follows:

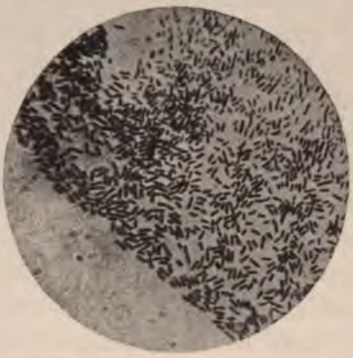


FIG. 73.—Bacillus coli communis. Impression-preparation from a colony on a gelatin-plate. (In comparing this photograph with that of the typhoid bacillus it must be remembered that Fig. 71 is much more highly magnified.)

1st. The typhoid bacillus is actively motile; the colon bacillus less actively, or slightly motile.

2d. The typhoid bacillus has numerous flagella which rise from all parts of the surface; the colon bacillus has a smaller number of flagella.

3d. In both, spore formation is absent.

4th. Both are decolorized by Gram's method.

5th. The colonies of the typhoid bacillus in gelatin develop more slowly than those of the colon bacillus.

6th. The appearance of superficial colonies in gelatin-plates.

7th. In media containing sugars the typhoid bacillus does not produce gas and the colon bacillus does produce gas.

8th. The typhoid bacillus produces an acid reaction without coagulation in milk, and the colon bacillus produces an acid reaction and coagulation.

9th. In peptone solutions the typhoid bacillus produces no indol, and the colon bacillus produces indol.

10th. The typhoid bacillus produces an invisible growth on potato, the colon bacillus a visible growth.

To these may be added the growth of the two organisms on special media like those of Wurtz, of Elsner and of Hiss, and the application of the serum reaction.

Injections of cultures of the bacillus coli communis into animals produce variable and uncertain results. Subcutaneous injection may lead to pus-formation; in rabbits and guinea-pigs injections may produce death apparently from poisons introduced.

Concerning the occurrence of the bacillus coli communis in the intestine of man, see page 123.

At autopsies on human subjects the great viscera are often found to have been infected by the colon bacillus, usually when some lesion of the intestine existed simultaneously, but in most cases without having produced much apparent damage to the organs invaded. The bacillus coli communis frequently occurs in mixed infections, as in wounds, inflammations and abscesses. It is often found in the peritoneum in peritonitis, and in the pus in appendicitis; it frequently occurs in the interior of gall-stones, with whose formation it may be connected.

There is a large number of more or less closely-related organisms which go by the name of the "*colon group.*" The limits of the colon group are extremely ill-defined.

Bacillus lactis aërogenes (Bacillus aërogenes).—A bacillus having a form similar to that of the colon bacillus, described as being larger and plumper. In the main its properties are similar to those of the colon bacillus. Its colonies are more circumscribed and elevated. It is said also to be non-motile. It coagulates milk more rapidly than the colon bacillus. It produces gas upon potato more rapidly than the colon bacillus, and more abundantly. It was described by Escherich, who also described the colon bacillus, assigning the bacillus lactis aërogenes rather to the upper part of the small intestine, and the colon bacillus to the lower portion. According to Kruse, the bacillus lactis aërogenes and its relatives differ from the bacillus coli communis chiefly in lacking motility.

Spirillum choleræ (Comma bacillus of cholera).—A rod- or staff-shaped organism, somewhat curved, hence the name "comma" bacillus. The curved forms, placed end to end, may produce an S-shaped body. The length is from .8 to 2 μ and the breadth from .3 to .4 μ. In cultures, genuine spirilla may be seen. In the whitish particles found in the stools of cases of cholera the organisms may be present in very large numbers. In these particles they may exhibit a very curious arrangement, lying parallel with one another, and, as remarked by Koch, they resemble a school of fish moving up stream. Involution forms, irregular in outline and staining poorly, are often seen in old cultures. The organism is motile, having a flagellum at one end. It does not form spores. It stains with the ordinary aniline dyes, but not by Gram's method. It is aërobic. It grows at the room temperature, but better in the incubator. On the ordinary media the growths are whitish. It grows best on neutral or alkaline media, and is very sensitive to a small amount of acid. It liquefies gelatin. The colonies on gelatin-plates have a very characteristic appearance. They are nearly round at first, and granular as seen under the low power of the microscope; but at the end of about twenty-four hours the outline is slightly irregular, and the surface looks as though it were covered with finely-broken glass. The outline later becomes still more irregular or scalloped. As liquefaction of the gelatin takes place a funnel-shaped depression is formed, into which the colony sinks. The plates should be kept at a temperature of from 20° to 22° C. In stab-cultures in gelatin a white growth

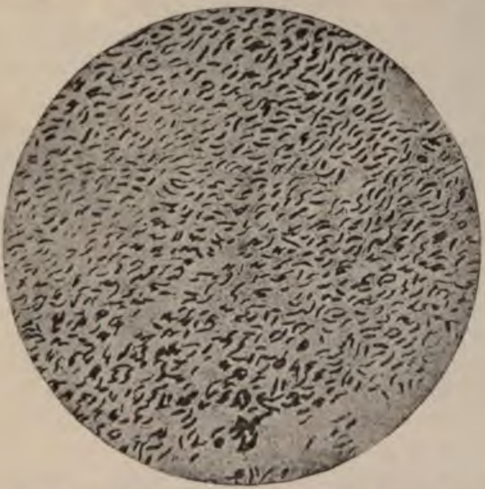
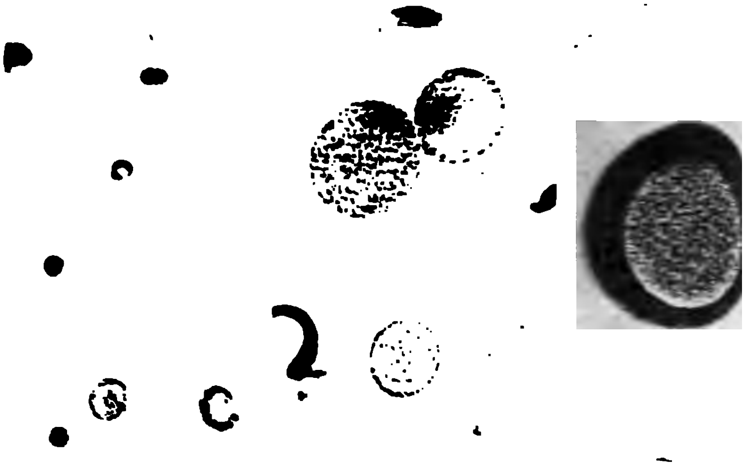


FIG. 74.—Spirillum of cholera, from a culture on starched linen. (Fränkel and Pfeiffer.)

[illegible]



[illegible] gelatin plates. Magnified [illegible] Forty-eight hours old. [illegible]

[illegible] bouillon a pellicle forms on [illegible] incubator the growth is whitis [illegible] elevated. Milk kept in th [illegible] in from thirty-six to forty-eigh [illegible] is finally coagulated. After growing it in Dun [illegible] peptone solution in the incubator, the addition [illegible] sulphuric acid develops a red color, owing to the formatio of indol and nitrites, the so-called "*cholera red*" reactior

The spirillum of cholera is said to be very sensitiv to drying, and, provided the drying be complete, is usually killed within twenty-four hours. It is killed in five minutes at a temperature of 65° C. It may retain its vitality in water for a long time; observations vary widely in respect to determining how long. In the ordinary food-substances it may survive long enough to allow them to act as carriers of the infection if eaten raw. The important fact is that the cholera spirillum is not a strict parasite, but under favorable conditions it may maintain its vitality for some time outside of the human body.

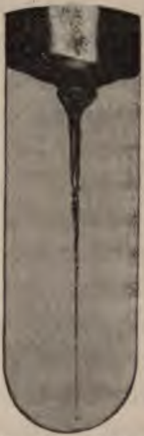


FIG. 76.—Spirillum of cholera, stab-culture in gelatin, two days old. (Fränkel and Pfeiffer.)

The animals ordinarily used for laboratory experiment are, in their normal condition, not susceptible to infection with the spirillum of cholera through the alimentary canal, and no animal is known which suffers from cholera excepting man, though a disease resembling cholera can be reproduced in animals when certain conditions are complied with. In particular it is necessary to avoid the influence of the acid gastric juice.

The following plan was adopted by Koch: The gastric juice was neutralized with a solution of sodium carbonate; the movements of the intestines were quieted by the injection of 1 c.c. of tincture of opium for each 200 grams of the body-weight; and a portion of a pure culture of the cholera spirillum was introduced into the stomach. When guinea-pigs were treated in this manner, in most cases a condition closely simulating cholera was produced. The animal died with symptoms of collapse. The small intestine contained a watery, flocculent fluid in which the spirilla of cholera were numerous. The mucous membrane of the intestine was swollen and reddened.

When mice or guinea-pigs receive an intra-peritoneal injection from a pure culture, death usually results, apparently, from the toxic substances contained in the culture. Pfeiffer has shown that by repeated doses, insufficient to kill the animal, of cultures whose vitality has been destroyed by heat or otherwise, the animal may be made immune. He has also shown that when living comma bacilli are introduced in the peritoneum of an immune animal they are rapidly destroyed and disintegrated (see page 146). He has advised the use of this reaction as a means of diagnosis, inasmuch as the spirilla which apparently resemble the spirillum of cholera, but are in reality different from it, do not become disintegrated when they are introduced in the peritoneum of an animal made immune to the spirillum of cholera. There is reason to think that blood from animals immune to the spirillum of cholera has an agglutinating action upon those bacteria like that seen when the blood-serum of cases of typhoid fever is mixed with living typhoid bacilli.

Although a positive demonstration that the spirillum of Koch is the cause of cholera is lacking, as far as the exact reproduction of the disease in animals is concerned, the necessary proof has been supplied by the accidental or intentional infection of laboratory investigators who were working with cholera, which has been known to occur on several occasions.

Bacteriological investigations of the victims of cholera have shown that the spirilla of cholera are present in very large numbers in the watery contents of the intestine, especially early in the disease. They appear in the lumina of the glands, and they may be seen underneath the epithelial cells. They may occur in the matters vomited. They usually are not found widely spread through the organs of the body. It is probable that the symptoms of the disease result from poisonous substances produced by the spirilla or contained in them.

The infection of cholera is usually transmitted through water, and numerous epidemics have been studied where the infection was traced to drinking-water, and the origin of the contamination was discovered. The infection may, however, be carried by other articles of food, and may be conveyed occasionally through contaminated clothing and bedding, and probably by flies. Although commoner in the summer-time, epidemics of cholera have been known to occur in the winter.

Bacteriological Diagnosis of Cholera.—When cases suspected of being cholera appear in a community, it becomes a matter of the utmost importance to determine the exact nature of the disease in order that it may not become epidemic. One of the first occasions when bacteriological methods were put into practice in the diagnosis of cholera was at the time of the appearance of that disease in the Port of New York in 1887.

According to Koch, the diagnosis may be made in twenty-four hours or less. It is important to obtain the discharges from the intestines as early in the course of the disease as possible, and while they are perfectly fresh. It may be necessary, however, to examine the moist dejecta on the linen or clothing, when no other material is available.

In the first place, one of the small, partly-solid particles which may be found in the discharges from the intestines should be smeared upon a cover-glass, fixed in the usual manner, stained with one of the aniline dyes, and examined with the microscope. If taken early in the disease, the comma bacilli will be present in large numbers, and they are likely to be arranged in more or less parallel groups (see above). If comma-shaped bacilli are found, a strong probability is created that the disease is Asiatic cholera. The motility of the organisms can be determined by ex amination in the hanging-drop. It is to be remembered that spirilla of various forms are common in the normal mouth, and may appear in the stools (see pages 121 and 177).

The diagnosis, however, should be confirmed by the use of culture-methods. Using the small, semi-solid particles from the intestinal discharges, gelatin-plates in the usual three dilutions (see page 74) should be made and kept at a temperature of 20° to 22° C. At the end of twenty-four hours or less the colonies of the spirillum of cholera should have been developed and should present the picture characteristic for these colonies in gelatin-plates (Fig. 75), which enables them to be differentiated from colonies of other bacteria. From one of these colonies, preparations may be made for microscopic examination, and a set of tubes may be inoculated. The most characteristic growth will be from stick-cultures in gelatin. The growth in Dunham's peptone solution may be tested for the development of indol and nitrites.

At the time that the first smear preparations and gelatin-plates are prepared, tubes of peptone solution should be inoculated directly from the intestinal contents, and kept in the incubator (Schottelius). After development has occurred, the production of indol may be tested by the addition of sulphuric acid. These tubes are especially valuable when unfavorable material or *when material containing small numbers of the spirilla is used.* In the incubator the spirilla may be expected to multiply in the peptone solution rapidly, and to appear upon the surface of the liquid in large numbers, even forming a visible film in six hours. Smears may be made from the surface part of these tubes, stained, and examined with a microscope. From the same material gelatin-plates should be prepared, and examined as soon as the colonies develop.

When cultures are obtained, their effects may be tested upon guinea-pigs by injecting them into the peritoneum.

The reaction described by Pfeiffer as resulting from the [illegible]ction of cholera spirilla into the peritoneum of immune [illegible]mals has been recommended as an additional means of [illegible]gnosis between the cholera spirillum and related forms.

In the examination of water for the spirillum of cholera, [illegible] 1 liter, or more, of water, add enough of a strong peptone solution to make it contain 1 per cent. peptone and [illegible] per cent. sodium chloride. (The strong peptone solution contains 20 per cent. peptone and 10 per cent. sodium chloride; is alkaline and sterile.) The water, with the peptone in it, is divided among a number of sterilized flasks. After twelve hours in the incubator, any vibrios in it are likely to have multiplied and to have formed a scum on the surface, which may be investigated for the characteristics of the spirillum of cholera according to the methods given above. See also page 113.

Since Koch's discovery of the cholera spirillum in 1883–84 a considerable number of bacteria have been described which resemble the cholera spirillum more or less closely, and which have to be taken into account in making examinations of material of any sort for it. This is particularly necessary in the investigation of water, in which such spirilla seem to occur quite frequently.

Vibrio Metschnikovi. — A comma-shaped organism, which though somewhat shorter and thicker may be very similar to the comma bacillus of cholera in form, and which, like it, may sometimes form genuine spirilla. It is motile and has a flagellum at one end. It does not form spores. It is aërobic. It stains with the aniline dyes, and is not stained by Gram's method. It grows at the room temperature. It liquefies gelatin somewhat more rapidly than the spirillum of cholera. The colonies on gelatin-plates are not all alike; some of them resemble those of vibrio proteus, and others are extremely like those of the spirillum of cholera. It grows upon the usual media. Blood-serum is liquefied by it. The growth on agar is grayish to yellowish, and abundant. It forms a pellicle on bouillon. In milk an acid reaction is developed with coagulation. In peptone solution it produces indol and nitrites like the spirillum of cholera. It is said to lead to the production of indol more intensely than the spirillum of cholera.

It is killed by a temperature of 50° C. in five minutes. It was discovered in chickens suffering from gastro-enteritis. It is pathogenic to chickens, pigeons and guinea-pigs, less so to mice and to rabbits. The comma-shaped organisms are found in the blood in guinea-pigs, pigeons and young chickens.

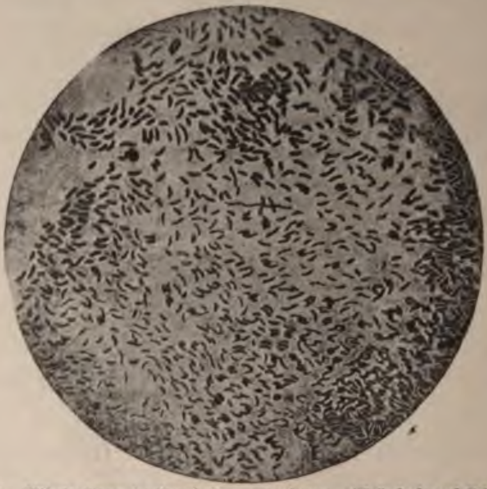


FIG. 77.—Vibrio Metschnikovi, from a culture. (Fränkel and Pfeiffer.)

Vibrio proteus (of Finkler and Prior).—A comma-shaped organism somewhat larger than the spirillum of cholera, sometimes exhibiting genuine spiral forms, and also, at times, involution forms. It is motile and has a flagellum at one end. It liquefies gelatin much more rapidly than the spirillum of cholera, and the colonies in gelatin develop more rapidly. At the end of twenty-four hours the colonies are uniformly circular, larger than those of the spirillum of cholera, and uniformly granular. On the other culture-media the growths are usually whitish. On potato it produces an abundant, moist, grayish-yellow deposit, and grows at the room temperature. It liquefies blood-serum; milk becomes acid. In peptone solution it does not form indol. It is less pathogenic to animals than the spirillum of cholera. It was supposed by its discoverers to be the cause of cholera nostras, but it appears to have no relation to that disease.

Spirillum Milleri.—A comma-shaped organism resembling vibrio proteus in many respects, and probably identical with it. In gelatin it grows more rapidly, and produces liquefaction more rapidly than the spirillum of cholera. On gelatin-plates, at the end of twenty-four hours, the colonies are uniformly circular and granular, lying in little depressions resulting from the liquefaction of the gelatin. Its growths in the other media are not characteristic. It liquefies blood-serum. It does not produce indol. It is less toxic to animals than the spirillum of cholera. It was isolated by Miller from a carious tooth.

See also Spirillum sputigenum, Part III.

Spirillum tyrogenum (of Deneke).—A comma-shaped organism not so large as the spirillum of cholera. It is motile, having a flagellum at one end. It does not form spores. In cultures, genuine spirilla may develop. Gelatin is liquefied more rapidly than by the spirillum of cholera, and the colonies develop more rapidly. The circumference of the colony is not perfectly round, and the surface may appear somewhat irregular. The colonies differ noticeably from the colonies of the cholera spirillum, in the more rapid liquefaction of gelatin. Milk containing litmus becomes acid, is subsequently decolorized, and is also coagulated. It liquefies blood-serum. It is said by some investigators not to form indol, and by others to do so occasionally. No pellicle forms in cultures upon bouillon. It is less toxic to animals than the spirillum of cholera. It was isolated originally from old cheese.

Vibrio Berolinensis.—A comma-shaped organism resembling the spirillum of cholera in form and in the position of its flagellum. It does not stain by Gram's method. It grows at the room temperature, but more rapidly in the incubator. The colonies upon gelatin, one or two days old, are decidedly more finely granular and more transparent than those of the spirillum of cholera, and the margin is almost absolutely smooth and circular. As the colonies become older they assume a more irregular and lobulated appearance, but are still more finely granular than the colonies of the cholera spirillum. Gelatin is very slowly liquefied. Its growth on the other culture-media is not remarkable. It forms indol in peptone solution, and it increases in the upper layers of the fluid. When guinea-pigs are inoculated in the peritoneal cavity, death occurs in one to two days. This organism was discovered in the water-supply of Berlin.

Other spirilla have been isolated from water by Günther (vibrio aquatilis in Spree water); by Dunbar from the Elbe River; by Russell from the Gulf of Naples; by Heider from the water of the Danube Canal; and in America, by Abbott, from the water of the Schuylkill (vibrio Schuylkiliensis); and many others have been described to which the limits of this work will not permit of allusion.

The **Spirillum** or **Spirochæta Obermeieri** (of relapsing fever).—A slim spirillum with numerous turns, 16 to 40 μ in length. The ends are pointed. It is actively motile. The spirillum is not stained by Gram's method but may be colored by the ordinary aniline dyes. The organism has never been cultivated. It is found abundantly in the blood and in the spleen during the attack of fever. The spleen is enlarged. The disease has been produced in apes by inoculating them with blood taken from men having the disease.

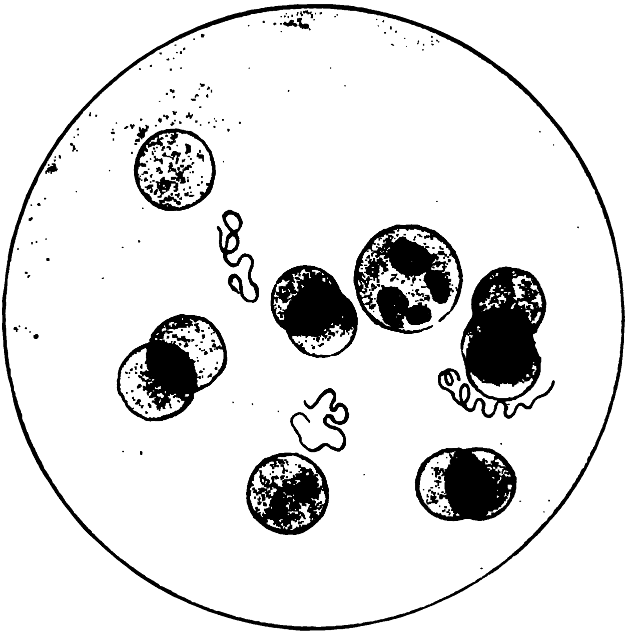


FIG. 78.—Spirillum of relapsing fever in the blood.

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